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(54) Title: SF-25 ANTIBODIES, ESPECIALLY CHIMERIC ANTIBODIES, WITH SPECIFICITY FOR THE HUMAN TUMOR SF-25 ANTIGEN, METHODS FOR THEIR PRODUCTION, AND USES THEREOF

(57) Abstract

The present invention concerns SF-25 monoclonal antibodies, especially chimeric antibodies, and derivatives and fragments thereof, having specificity to the SF-25 antigen of human tumor cells, methods of their production, pharmaceutical compositions containing them, and uses therefor.

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TITLE

SF-25 ANTIBODIES, ESPECIALLY CHIMERIC ANTIBODIES, WITH SPECIFICITY FOR THE HUMAN TUMOR SF-25 ANTIGEN, METHODS FOR THEIR PRODUCTION, AND USES THEREOF

I. Cross Reference to Related Applications

This application is related to United States Patent Applications Serial Nos.: 07/765,350, 07/765,351 and 07/765,612, all three of which were filed on September 25, 1991; 07/203,198 filed on June 7, 1988; and 07/130,777 (now abandoned) which was filed on December 9, 1987.

II. Field of the Invention

The present invention relates to SF-25 antibodies, especially chimerized antibodies, and fragments and derivatives thereof, which have specificity for the human tumor SF-25 antigen and especially to humanized chimeric SF-25 antibodies; to methods of producing these SF-25 antibodies, fragments, and derivatives thereof, using recombinant DNA technology; to the nucleotide and protein sequences coding for these SF-25 antibodies, fragments, and derivatives; to methods of obtaining and manipulating these sequences; processes for the manufacture of pharmaceutical compositions containing these SF-25 antibodies, fragments and derivatives thereof; and also the uses thereof.

III. Background of the Invention

A. Monoclonal Antibodies

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $F(ab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the free end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assays, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realization of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies (MAb) of defined and exquisite specificity (Kohler *et al., Nature 265*:295-497 (1975)).

MAbs produced from hybridomas are already widely used in basic research, are being tested in the treatment of human diseases, including cancer, viral and microbial infections, other diseases and disorders of the immune system. However, most MAbs have been produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the rodent MAb and will either remove it entirely or at least reduce its

effectiveness. In practice, MAbs of rodent origin may not be used in a patient for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Since MAbs of rodent origin are distinguished from human proteins, they are cleared rapidly from the human circulation. In addition, most mouse MAbs are not cytocidal against neoplastic cells in humans because the murine MAbs do not participate in human complement or cell-mediated cytotoxicity (Waldmann, T.A., *Science 252*:1657-1662 (1991); Robinson *et al., Hum. Antibod. Hybridomas 2*:84-93 (1991); Shin, S.U., *Biother. 3*:43-53 (1991); Ahmad *et al., Mol. Biother. 2*:67-73 (1990)).

Technology to develop MAbs that can circumvent these particular problems has met with a number of obstacles. This is especially true for MAbs directed to human tumor antigens that have been developed for the diagnosis and treatment of cancer. Since many human tumor antigens are not recognized as foreign by the human immune system, they probably lack immunogenicity in man. In contrast, those human tumor antigens that are immunogenic in mice can be used to induce murine MAbs which specifically recognize the human antigen, and which may also have therapeutic utility in humans, but have the previously referred to limitations.

Proposals have therefore been made for making non-human MAbs less antigenic and more cytocidal in humans. Such techniques can be generically termed "humanization" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

In particular, one procedure which has been proposed for the preparation of humanized antibodies is the so-called chimerization procedure.

Such chimerization procedures involve production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Some early methods for carrying out such a chimerization procedure are described in EP-A-0120694 (Celltech Limited), EP-A-0125023

(Genentech Inc. and City of Hope), EP-A-01714906 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University), and EP-A-0194276 (Celltech Limited). The latter Celltech application also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin derived protein in place of the Fc portion of the human immunoglobulin.

Most human MAbs obtained *in vitro* are of the IgM class or isotype which makes them difficult to purify and limits their *in vivo* application (Shin, S.U. *Biother.* 3:43-53 (1991)). To obtain human MAbs of the IgG isotype, it has been necessary to use complex techniques (e.g. cell sorting) to first identify and then isolate those few cells producing IgG antibodies. A need therefore exists for an efficient way to switch antibody classes at will for any given antibody of a predetermined or desired antigenic specificity.

The present invention bridges both the hybridoma and genetic engineering technologies to provide a quick and efficient method, as well as products derived therefrom, for the production of a chimeric human/non-human antibody which recognizes the SF-25 antigen.

The chimeric antibodies of the present invention embody a combination of the advantageous characteristics of MAbs derived from mouse-mouse hybridomas and of human MAbs. The chimeric MAbs, like murine MAbs can recognize and bind to the human SF-25 antigen; however, unlike murine MAbs the species-specific properties of the chimeric antibodies will avoid the inducement of harmful hypersensitivity reactions and will allow for resistance to clearance when used in humans *in vivo*. Moreover, using the methods disclosed in the present invention, any desired antibody isotype can be conferred upon a particular antigen combining site. The chimeric antibodies of the present invention may be useful in the diagnosis and treatment of cancer which express the SF-25 antigen in humans and other animals.

B. Colorectal Cancer and Hepatic Metastases

Some tumors and other diseased tissues selectively express constitutive antigens which are not expressed by normal animal tissues. An example is colorectal adenocarcinoma and its SF-25 constitutive antigen.

Colorectal cancer is one of the most common malignancies in both men and women in the Western world. More than 150,000 new cases will be diagnosed in 1991 in the United States alone (Boring *et al., Cancer Statistics 41*:19-36 (1991)). Despite major advances in general patient care and surgical therapy, the mortality rate associated with this disease has not changed significantly over the last forty years (Fleischer *et al., JAMA 261*:580-586 (1989)). Indeed, about 60,000 patients die of this disease each year in this country principally because of advanced disease or recurrence (Cancer Facts & Figures - 1990, *American Cancer Society, Inc.*, Atlanta, Ga. (1990)).

Sixty percent of the patients with advanced colon adenocarcinoma will develop hepatic metastases (Weiss, L., *J. Pathol. 150*:195-203 (1986)). Although 70-80% of these patients will present with operable tumors at the time of diagnosis, even complete surgical resection is often unable to permit long term survival due to the presence of occult disease or hepatic micrometastases. Numerous post operative adjuvant treatment regimens have failed to reduce the incidences of hepatic metastases and tumor recurrence (Grem, J.L., *Semin. Oncol. 18 (Suppl. 1)*:17-26 (1991); Buyse *et al., JAMA 259*:3571-3578 (1988); Mayer, R.J., *N. Engl. J. Med. 322*:399-401 (1990); Wolmark *et al., J. Natl. Cancer Inst. 80*:30-36 (1988)). The death rate will undoubtedly remain the same until improved methods for the treatment of hepatic involvement become available. Thus, new, useful clinical treatment regimens are needed.

C. The SF-25 Antigen

A repertoire of MAbs against transformed cells of human endodermal origin has been developed (Wilson *et al., Proc. Natl. Acad. Sci. 85*:3140-3144 (1988)). One such murine MAb, SF-25, recognizes a 125 Kd cell surface glycoprotein designated the SF-25 antigen (Takahashi *et al., Cancer Res. 48*:6573-6579 (1988)). The hybridoma which secretes the SF-25 MAb was deposited under the provisions of the Budapest Treaty at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, and has been given the ATCC designation HB 9599.

Murine MAb SF-25 recognizes the SF-25 antigen which is highly expressed in human colon adenocarcinomas, their hepatic metastases, and other primary tumors of endodermal origin. Murine MAb SF-25 has been immunolocalized in tumor xenografts in nude mice (Takahashi *et al., Cancer Res.48*:6573-6579 (1988); Takahashi *et al., Gastroenterol. 96*:1317-1329 (1989)). Furthermore, this antibody has been shown to be useful for the immunolocalization of colon adenocarcinoma *in vivo* (Id.).

Metabolic and cell surface labeling studies have demonstrated that the SF-25 antigen is a disulfide-bond-linked heterodimer which is composed of two glycosylated subunits termed α and β. The expression of the SF-25 antigen in colon adenocarcinoma tissues is uniform in contrast to the heterogeneous expression of other tumor associated antigens (Atkinson *et al., Cancer Res. 42*:4820-4823 (1982); Hand *et al., Id. 43*:728-735 (1983)). In addition, it appears that there is no antigenic modulation of the SF-25 antigen in liver metastases (Takahashi *et al.*, submitted to *Cancer Res.*). The SF-25 antigen and antibodies which recognize this antigen have been extensively described in related U.S. Patent Application Serial Numbers 07/203,198 and 07/130,777 which were filed on June 7, 1988 and December 9, 1987, respectively, the contents of which are herein incorporated by reference.

The SF-25 antigen is a constitutive antigen that is expressed on most if not all tumors of endodermal origin. The SF-25 antigen has been shown by

immunohistological staining to be expressed by the following human tumor types: colon adenocarcinoma; rectal adenocarcinoma; hepatocellular carcinoma; cholangiocellular carcinoma; gastric adenocarcinoma; breast adenocarcinoma; pancreatic adenocarcinoma; bladder adenocarcinoma; squamous cell carcinoma of the lung;adenocarcinoma of the lung; small cell carcinoma of the lung; large cell carcinoma of the lung; kidney carcinoma; ovary adenocarcinoma; cervix carcinoma; endometrial adenocarcinoma; choriocarcinoma; leukemia; lymphoma; and malignant melanoma. Previous studies of the SF-25 antigen's distribution revealed that a number of normal tissues were found to be negative by immunohistological staining including: esophagus; stomach; small intestine; colon; liver; bile duct; spleen; adrenal gland; lung; thyroid; skin; skeletal muscle; myocardium; connective tissue; brain; and spinal cord. Positive staining was present in a subpopulation of proximal tubular cells of the kidney. Weak staining was also observed in normal islet cells of the pancreas.

The SF-25 antigen is localized on the tumor cell surface and antibody binding to the SF-25 antigen does not induce internalization therefrom. The SF-25 antigen is not shed from the cell when it is examined by radioimmunoassay in culture supernatant and flow cytometric analysis (Takahashi *et al., Cancer Res. 48*:6573-6579 (1988)). Furthermore, MAb SF-25 has a high association constant (K_A = 1.36 x 10^8/M) and is able to immunolocalize to human colon adenocarcinomas established in the livers of nude mice (Takahashi *et al., Gastroenterology 96*:1317-1329 (1989)). The high number of antibody binding sites per cell (2.5 x 10^5/colon adenocarcinoma cell) suggest that the SF-25 MAb will be bound to the tumor cells in a high density. Taken together, these properties suggest that the SF-25 MAb may be effective as an immunotherapeutic reagent (Schlom *et al.*,in *Monoclonal Antibodies in Cancer: Advances in Diagnosis and Treatment*, (Roth, J.A. Ed.), Futura Publishing Company, Mount Kisco, NY, 1-65 (1986); Oldham, R.K., *in Biological Response Modifiers and Cancer Therapy*, (Chlao, J.K., Ed.) Marcel Dekker, Inc. New York, 3-16 (1988)).

D. Monoclonal Antibodies and the Treatment of Cancer Patients

Possible anti-tumor mechanisms mediated by MAbs include: 1) induction of tumor cytotoxicity by effector cells such as NK-cells and macrophages (Ravetch *et al., Ann. Rev. Immunol. 9*:457-492 (1991)); 2) activation of complement and induction of complement-mediated cytotoxicity (Frank, M. M., *N. Engl. J. Med. 316*:1525-1530 (1987)); 3) interference with cell growth or differentiation by binding growth factors or receptors on the surface of tumor cells (Sporn *et al., Nature 313*:745-747 (1985)); Rodeck *et al., Cancer Res. 47*:3692-3696 (1987)); 4) induction of anti-idiotypic antibodies which subsequently have been used as novel vaccines against tumors (Wettendorff *et al., Proc. Natl. Acad. Sci. USA 86*:3787-3791 (1989)); and 5) delivery of cytotoxic agents such as drugs, toxins, and radionucleotides to the tumor cells (Vitetta *et al., Science 238*:1098-1104 (1987); Waldmann, T.A., *Science 252*:1657-1662 (1991); Dillman, R.O., *Ann. Int. Med. 111*:592-603 (1989)).

Cells with cytotoxic potential that bear receptors for the Fc fragment of IgG (FcγR) may bind and lyse target cells in the presence of antibody (antibody-dependent cell-mediated cytotoxicity; ADCC) (Kay *et al., J. Immunol. 118*:2058-2066 (1977); Lubeck *et al., Cell. Immunol. 111*:107-117 (1988)). ADCC requires the simultaneous binding of the Fab fragment of the antibody to its antigen and the binding of the Fc fragment to FcγR expressed on the effector cells. Macrophages express the three types of the FcγR which have been identified in human cells (FcγRI, II and III). FcγRI is found only on macrophages and is important for ADCC. NK-cells only express low affinity FcγR type III which will initiate ADCC by NK-cells upon binding to antibody (Ravetch *et al., Ann. Rev. Immunol. 9*:457-492 (1991); Unkeless *et al., Id. 6*:251-281 (1988); Adams *et al., Id. 2*:283-318 (1984); Perussia *et al., J. Exp. Med. 170*:73-86 (1989); Vivier *et al., J. Immunol. 146*:206-210 (1991)).

The initial use of unmodified murine MAbs to treat humans with cancer has been disappointing. Only 23 partial and 3 complete remissions have been reported among 185 patients in 25 clinical trials. This is partially due to the fact that most mouse MAbs are not cytocidal against neoplastic cells in humans because these MAbs do not participate in human-complement or cell-mediated cytotoxicity (Waldmann, T.A., *Science 252*:1657-1662 (1991); Catane *et al.*, *J. Med. Sci. 24*:471 (1988)).

A chimeric MAb (c-SF-25 MAb) that has the Fc fragment of human IgG1 and the Fab fragment of the murine SF-25 MAb has been mentioned in Takahashi *et al.*,*Hepatology A12*:915 (1990); Takahashi, *et al. J. Cell. Biochem. Suppl. 15*:Part E pg. 139 (1991); Takahashi *et al.*, *Immunocon. Radiopharm. 4*:208 (1991); *Id.*, p. 237) (Takahashi *et al.*, *Antibody Immunocon. Radiopharm. 3*:86 (1990)). This chimeric construct induces ADCC by both human NK-cells and macrophages *in vitro*, since the FC fragment of human IgG1 interacts with FcγRI and also with FcγR type III.

IV. Summary of the Invention

The present invention provides a polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human immunoglobulin chain, both said sequences in operable linkage with each other.

The present invention also provides a host transformed with a polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human

immunoglobulin chain, both said sequences in operable linkage with each other.

The present invention also provides a eukaryotic or prokaryotic host transfected with a polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human immunoglobulin chain, both said sequences in operable linkage with each other.

The present invention also provides a chimeric immunoglobulin heavy chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

The present invention also provides a chimeric immunoglobulin light chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

The present invention also provides a chimeric antibody molecule comprising two light chains and two heavy chains, each of said chains comprise at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody given the ATCC designation HB 9599, or a fragment or derivative of said chimeric antibody.

The present invention also provides a process for preparing a chimeric immunoglobulin heavy chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599,comprising culturing a host capable

of expressing said heavy chain under culturing conditions; expressing said heavy chain; and recovering said heavy chain from said culture.

The present invention also provides a process for preparing a chimeric immunoglobulin light chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising culturing a host capable of expressing said light chain under culturing conditions; expressing said light chain; and recovering said light chain from said culture.

The present invention also provides a process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the hybridoma cell line given the ATCC designation HB 9599 comprising culturing a host capable of expressing said light chain under culturing conditions, expressing said light chain, and recovering said light chain from said culture; separately culturing a host capable of expressing said heavy chain under culturing conditions, expressing said heavy chain, and recovering said heavy chain from said culture; and associating said recovered heavy chain and light chain, thereby preparing said chimeric immunoglobulin, fragment or derivative.

The present invention also provides a process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising co-culturing a host capable of expressing said heavy chain with a host capable of expressing said light chain under culturing conditions; expressing said heavy chain and said light chain; permitting said heavy chain and said light chain to

associate into said chimeric immunoglobulin, fragment or derivative; and recovering said chimeric immunoglobulin, fragment or derivative from said culture.

The present invention also provides a process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the hybridoma cell line given the ATCC designation HB 9599, comprising culturing a host capable of expressing said heavy chain and said light chain under culturing conditions; expressing said chimeric immunoglobulin, fragment or derivative and recovering from said culture said chimeric immunoglobulin, fragment or derivative.

The present invention also provides an immunoassay method for detecting an antigen capable of binding to the chimeric SF-25 monoclonal antibody in a sample comprising contacting said sample with the detectably labeled antibody, fragment or derivative thereof; and detecting said antigen by detecting the binding of said antigen to said antibody, fragment or derivative.

The present invention also provides an imaging method for detecting a tissue antigen capable of binding to the chimeric SF-25 monoclonal antibody, comprising contacting the detectably labeled antibody, fragment or derivative thereof with said tissue; and detecting said antigen.

The present invention also provides a process for the manufacture of a pharmaceutical composition for use in the killing of cells expressing an antigen, which antigen is capable of binding to the chimeric SF-25 monoclonal antibody, comprising as an active ingredient an effective dose of the antibody, fragment or derivative thereof.

The present invention also provides a process for the manufacture of a pharmaceutical composition for use in treating an animal having a tumor expressing an antigen, which antigen is capable of binding to the chimeric SF-25 monoclonal antibody, comprising as an active ingredient an effective dose

of the antibody, fragment or derivative thereof. The pharmaceutical compositions of the present invention can be used to treat animals with the following tumors: colon adenocarcinoma; hepatocellular carcinoma; cholangiocellular carcinoma; gastric adenocarcinoma; rectal adenocarcinoma; breast adenocarcinoma; bladder adenocarcinoma; squamous cell carcinoma of the lungs; adenocarcinoma of the lungs; large cell carcinoma of the lungs; small cell carcinoma of the lungs; lymphoproliferative disease; myeloproliferative disease; lymphoma; leukemia; kidney carcinoma; ovary adenocarcinoma; cervical carcinoma; uterine endometrial adenocarcinoma; liver hepatoma; choriocarcinoma; malignant melanoma, as well as pancreatic adenocarcinoma and other pancreatic cancers. Previous studies of the SF-25 antigen's distribution revealed that a number of normal tissues were found to be negative by immunohistological staining including: esophagus; stomach; small intestine; colon; liver; bile duct; spleen; adrenal gland; lung; thyroid; skin; skeletal muscle; myocardium; connective tissue; brain; and spinal cord. Positive staining was present in a subpopulation of proximal tubular cells of the kidney. Weak staining was also observed in normal islet cells of the pancreas.

The present invention also provides for the use of an effective dose of an SF-25 antibody or fragment or derivative thereof, including a chimeric SF-25 monoconal antibody, for killing cells expressing an antigen, which antigen is capable of binding to said SF-25 antibody.

The present invention also provides for the use of an effective dose of an SF-25 antibody, or fragment or derivative thereof, including a chimeric SF-25 monoclonal antibody, for treating an animal having a tumor expressing an antigen, which antigen is capable of binding to said SF-25 antibody.

The present invention also provides for the use of an effective dose of an SF-25 antibody or fragment or derivative thereof, including a chimeric SF-25 monoclonal antibody, for treating an animal which has pancreatic carcinoma or other pancreatic cancer, which expresses an antigen, which antigen is capable of binding to said SF-25 antibody.

The present invention also provides for the use of an effective dose of an SF-25 antibody, or fragment or derivative thereof, including a chimeric SF-25 monoclonal antibody for treating an animal having cancer which expresses an antigen, which antigen is capable of binding to said SF-25 antibody, with the cancer selected from the group consisting of colon adenocarcinoma, hepatocellular carcinoma, cholangiocellular carcinoma, gastric adenocarcinoma, rectal adenocarcinoma, breast adenocarcinoma, bladder adenocarcinoma, squamous cell carcinoma of the lungs, adenocarcinoma of the lungs, large cell carcinoma of the lungs, small cell carcinoma of the lungs, lymphoproliferative disease, myeloproliferative disease, lymphoma, leukemia, kidney carcinoma, ovary adenocarcinoma, cervical carcinoma, uterine endometrial adenocarcinoma, liver hepatoma, choriocarcinoma, malignant meloma and pancreatic carcinoma, including the primary tumors or metastases or micrometastases of these diseases.

The present invention also provides a method of killing cells expressing an antigen, which antigen is capable of binding to an SF-25 antibody, including a chimeric SF-25 monoclonal antibody, comprising delivering to said cells an effective dose of the antibody, fragment or derivative thereof, and allowing said killing to occur.

The present invention also provides a method of treating an animal suspected of having a tumor expressing an antigen which is capable of binding to an SF-25 antibody, especially a chimeric SF-25 monoclonal antibody, comprising administering to said animal an effective dose of the antibody, fragment or derivative thereof.

The present invention also provides a method of treating a cancer in an animal which comprises administering an effective dose of an antibody specific

to the SF-25 antigen, especially a chimeric SF-25 antibody, or a derivative or fragment thereof, to said animal having a cancer selected from the group consisting of colon adenocarcinoma, hepatocellular carcinoma, cholangiocellular carcinoma, gastric adenocarcinoma, rectal adenocarcinoma, breast adenocarcinoma, bladder adenocarcinoma, squamous cell carcinoma of the lungs, adenocarcinoma of the lungs, large cell carcinoma of the lungs, small cell carcinoma of the lungs, lymphoproliferative disease, myeloproliferative disease, lymphoma, leukemia, kidney carcinoma, ovary adenocarcinoma, cervical carcinoma, uterine endometrial adenocarcinoma, liver hepatoma, choriocarcinoma, malignant meloma and pancreatic carcinoma, including the primary tumors or metastases or micrometastases of these diseases.

V. Brief Description of the Figures

Figure 1. Restriction maps of the germline J_K region and the 3.2 Kb HindIII fragment containing the functionally rearranged SF-25 V_K gene. The bracket above the germline restriction map indicates the J_K probe used (Figure 1A). Restriction maps of the germline J_H region and the 4.5 Kb EcoR1 fragment containing the functionally rearranged SF-25 V_H gene (Figure 1B). Exons are represented with solid boxes. The bracket above the germline restriction map indicates the J_H probe used. EcoR1 (E), HindIII (H), Xbal (X), and BamH1 (B) restriction enzyme sites are shown. "E" corresponds to the enhancer element present in the mouse H chain intron. "L" indicates the exon containing the leader peptide sequence. The L chain enhancer is provided by the L chain expression vector.

Figure 2. Structure of the SF-25 chimeric L and H chain expression vectors. The SF-25 L chain expression vector containing the xanthine-guanine phosphoribosyl (gpt) gene (Figure 2A). The SF-25 H chain expression vector containing the human Igγ1 constant region (Figure 2B).

Figure 3. Competitive inhibition of ^{125}I-labeled c-SF-25 MAb binding to LS 180 cells by unlabeled chimeric and murine SF-25 MAb. The binding of ^{125}I-labeled c-SF-25 MAb was inhibited by both chimeric and murine SF-25 MAb. In contrast, this binding was not inhibited by an unlabeled non-relevant MAb (B2TT). These results demonstrate that c-SF-25 MAb recognizes the same epitope as the murine MAb.

Figure 4. Antibody dependent cell-mediated cytotoxicity (ADCC) mediated by human effector cells in a 4 hour ^{51}Cr-release assay using LS 180 as the target cell. c-SF-25 MAb induced ADCC but the murine SF-25 MAb did not produce this activity by human PBLs (Figure 4A). Both purified NK-cells and macrophages mediated strong ADCC activity in the presence of c-SF-25 MAb (Figure 4B).

Figure 5. All control animals developed multiple "cannon ball-like" hepatic metastases of human colon adenocarcinomas (Figure 5A). A representative liver of an animal treated with c-SF-25 MAb which is free of detectable disease (Figure 5B). The $F(ab')_2$ fragment of c-SF-25 MAb had little effect on the degree of hepatic metastases (Figure 5C) and all mice developed hepatic tumors similar to controls.

Figure 6. ADCC mediated by murine effector cells in an 8 hour ^{51}Cr-release assay. Thioglycolate-elicited murine macrophages mediated ADCC against LS 180 cells in the presence of c-SF-25 MAb (Figure 6A). Murine splenocytes (Figure 6B) and murine NK-cells purified from these splenocytes (Figure 6C) also exhibited ADCC activity against LS 180 cells in the presence of c-SF-25 MAb.

Figure 7. Survival curves of mice treated with c-SF-25 MAb. All mice treated with c-SF-25 MAb survived more than six weeks, whereas all control mice died within six weeks after LS 180 cell injection. The survival rate of c-SF-25 MAb-treated animals was significantly longer than that of controls ($p < 0.0002$).

Figure 8. ADCC produced in the presence of c-SF-25 MAb (20 μg/ml) by murine splenocytes. Cytotoxicity was studied using LS 180 human colon adenocarcinoma cells at different effector to target (E:T) ratios.

Figure 9. Direct binding of c-SF-25 and MAb to LS 180 cells at different temperatures. The specific binding of ^{125}I-labeled c-SF-25 MAb increased as the temperature increased from 4°C to 37°C.

Figure 10. Direct binding of ^{125}I-labeled GA733 and 323/A3 MAbs to LS 180 tumor cells. GA733 and 323/A3 MAbs showed high binding to LS 180 cells both at 4°C and at 37°C.

Figure 11. ADCC mediated by murine NK-cells and macrophages at an E:T ratio of 50 to 1 in the presence of different concentrations of MAbs. ADCC activity by murine splenocytes was induced by c-SF-25 at all antibody concentrations tested (Figure 11A). Chimeric SF-25 MAb induced similar ADCC by macrophages (Figure 11B).

VI. Definitions

In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided. Any terms which are not specifically defined in this or other sections of this patent application have the ordinary meaning they would have when used by one of skill in the art to which this invention applies.

As used herein, an effective derivative or fragment of an antibody means a derivative or fragment of an antibody which is still capable of selectively binding to the same molecule(s) as that which the whole antibody binds to.

As used herein, a constitutive antigen means an antigen that is produced by the majority or all of the cells of a particular tumor type or disease type.

As used herein, imaging means the visualization or location of cells, or tumors, or other diseased tissues which express constitutive antigens and which bind detectably labeled, targeted, cytotoxic, effector cells to them.

As used herein, a detectable label is an atom or molecule which is attached to the targeted, cytotoxic, effector cell or constituent thereof, and which is used in imaging cells or tumors or other diseased tissues. Examples of such labels include, but are not limited to, radioisotopic labels, non-radioactive isotopic labels, chemiluminescent labels, fluorescent labels and enzyme labels.

VII. Description of the Preferred Embodiments

The present invention derives from the discovery of a chimerized SF-25 antibody with specificity for the human tumor SF-25 antigen which is constitutively expressed by human colon adenocarcinoma cells and other human tumors of endodermal origin. The present invention also derives from a method to produce these chimerized SF-25 antibodies and from methods to use them diagnostically and therapeutically.

In the present application, the term "chimeric antibody molecule" is used to describe an antibody molecule having heavy and/or light chains comprising at least the variable regions of heavy and/or light chains derived from one immunoglobulin molecule linked to at least part of a second protein. The second protein may comprise additional antibody constant region domains derived from a different immunoglobulin molecule or a non-immunoglobulin protein. The term "humanized chimeric antibody molecule" is used to describe a molecule having heavy and light chain variable region domains derived from an immunoglobulin from a non-human species, the remaining immunoglobulin constant region domains of the molecule being derived from a human immunoglobulin.

A. Production of the Chimeric SF-25 Monoclonal Antibody

1. SF-25 Antibodies and Antibody Fragments

In the following description, reference will be made to various methodologies well-known to those skilled in the art of immunology. Standard reference works setting forth the general principles of immunology include the work of Klein, J. (*Immunology: The Science of Cell-Noncell Discrimination*, John Wiley & Sons, New York (1982)); Kennett *et al.* (*Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses*, Plenum Press, New York (1980)); Campbell, A. ("Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon *et al.*, eds.), Elsevier, Amsterdam (1984)); and Eisen, H.N., (In: *Microbiology*, 3rd Ed. (Davis *et al.*, Harper & Row, Philadelphia (1980)).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. As used herein, the term "hapten" is intended to refer to any molecule capable of being bound by an antibody. The term "epitope" is meant to refer to that portion of a hapten which can be recognized and bound by an antibody. A hapten or antigen may have one, or more than one epitope. An "antigen" is a hapten which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. The specific reaction referred to above is meant to indicate that the hapten will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" (Ab) or "monoclonal antibody" (Mab) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of binding a hapten. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody,

clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)).

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the SF-25 antigen can be administered to an animal in order to induce the production of sera containing polyclonal antibodies that are capable of binding the SF-25 antigen. In a preferred method, a preparation of SF-25 antigen is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or hapten binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al., Nature 256*:495 (1975); Kohler *et al., Eur. J. Immunol. 6*:511 (1976); Kohler *et al., Eur. J. Immunol. 6*:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with SF-25 antigen or, more preferably, with an SF-25-expressing cell. Although any such cell may be employed in accordance with the present invention, it is preferable to employ the hepatocellular carcinoma cell line, FOCUS (Lun *et al., In Vitro 20*:493-504 (1984)). Suitable cells can be recognized by their capacity to bind anti-SF-25 antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at 56°C), and supplemented with 10 μg/l of nonessential amino acids, 1,000 U/ml of penicillin, and 100 μg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP_2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybri-

doma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterology 80*:225-232 (1981), which reference is herein incorporated by reference). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the SF-25 antigen. A preferred hybridoma cell line, obtained by this process, is the monoclonal antibody-producing cell line "SF-25." This cell line produces monoclonal antibody "SF-25" which is capable of binding to the SF-25 antigen. Cell line "SF-25" was deposited under the provisions of the Budapest Treaty with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, on December 8, 1987, and given the ATCC designation: HB 9599.

Through application of the above-described methods, additional cell lines capable of producing antibodies which recognize epitopes of the SF-25 antigen can be obtained.

Alternatively, additional antibodies capable of binding to the SF-25 antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, antibodies capable of binding the SF-25 antigen are used to immunize an animal. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce antibody whose ability to bind to anti-SF-25 antibody can be specifically blocked by the SF-25 antigen. Such antibodies comprise anti-idiotypic antibodies to the anti-SF-25 antibody. Such antibodies can be used to immunize an animal, and thereby induce the formation of anti-SF-25 antibodies. Since anti-idiotypic antibodies can be used to immunize an animal and thus provoke the production of anti-SF-25 antibodies, they provide one method for inducing, or enhancing, an animal's immune response to colon cancer.

It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibody of the present invention may be used according to the methods

disclosed herein for the detection and treatment of colon adenocarcinoma in the same manner as intact antibody. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). Alternatively, hapten-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

2. Production of the Chimeric SF-25 MAb by Recombinant Technology

The identification of the amino acid sequence of the c-SF-25 MAb or fragment of derivatives thereof, permits these molecules to be produced through the application of recombinant DNA techniques. For example, an oligonucleotide can be constructed which is capable of encoding the c-SF-25 MAb or fragment or derivative thereof. Such an oligonucleotide can be operably linked into an expression vector and introduced into a host cell to enable the expression of the c-SF-25 MAb or fragment or derivative thereof by that cell. Techniques for synthesizing such oligonucleotides are disclosed by, for example, Wu *et al.*, *Prog. Nucl. Acid. Res. Molec. Biol. 21*:101-141 (1978)). Procedures for constructing recombinant molecules in accordance with the above-described method are disclosed by Maniatis *et al.*, In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1984), and also in *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press, (Sambrook *et al.*, Eds.) Cold Spring Harbor, NY (1989) which references are herein incorporated by reference.

The identification of the amino acid sequence of the c-SF-25 MAb, or fragments of this MAb, also permits the cloning of the gene which encodes the c-SF-25 MAb.

Any of a variety of methods may be used to clone the c-SF-25 MAb gene. One such method entails analyzing a shuttle vector library of cDNA inserts (derived from an c-SF-25 MAb expressing cell) for the presence of an insert which contains the c-SF-25 MAb gene. Such an analysis may be

conducted by transfecting cells with the vector, and then assaying for c-SF-25 MAb expression. A preferred method for cloning the c-SF-25 MAb gene entails determining the amino acid sequence of the c-SF-25 MAb molecule. Although it is possible to determine the entire amino acid sequence of the c-SF-25 MAb molecule it is preferable to determine the sequence of peptide fragments of the molecule. If the peptides are greater than 10 amino acids long, this sequence information is generally sufficient to permit one to clone a gene such as the gene for the c-SF-25 MAb molecule.

To accomplish this task, c-SF-25 MAb molecules are preferably purified from producer cells by monoclonal antibody affinity chromatography and isolated by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis ("SDS-PAGE") and electroelution. The c-SF-25 MAb molecules are fragmented as with cyanogen bromide, or with proteases such as papain, chymotrypsin, trypsin, etc. (Oike *et al., J. Biol. Chem. 257*:9751-9758 (1982); Liu *et al., Int. J. Pept. Protein Res. 21*:209-215 (1983)). The resulting peptides are separated, preferably by reverse-phase HPLC, and subjected to amino acid sequencing. To accomplish this task, the protein is preferably analyzed by automated sequencers.

Once one or more suitable peptide fragments have been sequenced, the DNA sequences capable of encoding them are examined. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid (Watson, J.D., In: *Molecular Biology of the Gene*, 3rd Ed., W.A. Benjamin, Inc., Menlo Park, CA (1977), pp. 356-357). Using the genetic code, one or more different oligonucleotides can be identified, each of which would be capable of encoding the c-SF-25 MAb peptides. The probability that a particular oligonucleotide will, in fact, constitute the actual c-SF-25 MAb-encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic cells. Such "codon usage rules" are disclosed by Lathe *et al., J. Molec. Biol. 183*:1-12 (1985). Using the "codon usage rules" of Lathe, a single oligonucleotide, or a set of

oligonucleotides, that contains a theoretical "most probable" nucleotide sequence capable of encoding the c-SF-25 MAb peptide sequences is identified.

Although occasionally an amino acid sequence may be encoded by only a single oligonucleotide, frequently the amino acid sequence may be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the members of this set contain oligonucleotides which are capable of encoding the peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is identical to the nucleotide sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the peptide.

The oligonucleotide, or set of oligonucleotides, containing the theoretical "most probable" sequence capable of encoding the SF-25 MAb fragment is used to identify the sequence of a complementary oligonucleotide or set of oligonucleotides which is capable of hybridizing to the "most probable" sequence, or set of sequences. An oligonucleotide containing such a complementary sequence can be employed as a probe to identify and isolate the c-SF-25 MAb gene (Maniatis *et al., Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982).

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the c-SF-25 MAb gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified (using the above-described procedure), synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing the SF-25 gene. Single stranded oligonucleotide molecules complementary to the "most probable" SF-25 peptide encoding sequences can be synthesized using procedures which are

well known to those of ordinary skill in the art (Belagaje *et al., J. Biol. Chem. 254*:5765-5780 (1979); Maniatis *et al.*, In: *Molecular Mechanisms in the Control of Gene Expression*, Nierlich *et al.*, Eds., Acad. Press, NY (1976); Wu *et al., Prog. Nucl. Acid Res. Molec. Biol. 21*:101-141 (1978); Khorana, R.G., *Science 203*:614-625 (1979)). Additionally, DNA synthesis may be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Maniatis *et al.* (In: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1982)) and by Haymes *et al.* (In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)), which references are herein incorporated by reference. The source of DNA or cDNA used will preferably have been enriched for SF-25 MAb sequences. Such enrichment can most easily be obtained from cDNA obtained by extracting RNA from cells, such as hybridoma cells, which produce high levels of SF-25. An example of such a cell is the hybridoma cell line given the ATCC designation HB 9599 which was previously described.

To identify and clone the gene which encodes the SF-25 protein, a DNA, or more preferably a cDNA, library is screened for its ability to hybridize with the oligonucleotide probes described above. Suitable DNA preparations (such as human genomic DNA) are enzymatically cleaved, or randomly sheared, and ligated into recombinant vectors. The ability of these recombinant vectors to hybridize to the above-described oligonucleotide probes is then measured. Vectors found capable of such hybridization are then analyzed to determine the extent and nature of the SF-25 sequences which they contain. Based purely on statistical considerations, a gene such as that which encodes the c-SF-25 molecule could be unambiguously identified (via hybridization screening) using an oligonucleotide probe having only 18 nucleotides.

Thus, in summary, the actual identification of c-SF-25 peptide sequences permits the identification of a theoretical "most probable" DNA sequence, or a set of such sequences, capable of encoding such a peptide. By

constructing an oligonucleotide complementary to this theoretical sequence (or by constructing a set of oligonucleotides complementary to the set of "most probable" oligonucleotides), one obtains a DNA molecule (or set of DNA molecules), capable of functioning as a probe to identify and isolate the c-SF-25 MAb gene.

Techniques such as, or similar to, those described above have successfully enabled the cloning of genes for human aldehyde dehydrogenases (Hsu *et al., Proc. Natl. Acad. Sci. USA 82*:3771-3775 (1985)), fibronectin (Suzuki *et al., Eur. Mol. Biol. Organ. J. 4*:2519-2524 (1985)), the human estrogen receptor gene (Walter *et al., Proc. Natl. Acad. Sci. USA 82*:7889-7893 (1985)), tissue-type plasminogen activator (Pennica *et al., Nature 301*:214-221 (1983)) and human term placental alkaline phosphatase complementary DNA (Kam *et al., Proc. Natl. Acad. Sci. USA 82*:8715-8719 (1985)).

In a alternative way of cloning the c-SF-25 MAb gene, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing MAb c-SF-25) into an expression vector. The library is then screened for members capable of expressing a protein which binds to SF-25 antigen, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as MAb c-SF-25, or fragments or derivatives thereof. In this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing c-SF-25 MAb. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment.

An "expression vector" is a vector which (due to the presence of appropriate transcriptional and/or translational control sequences) is capable of expressing a DNA (or cDNA) molecule which has been cloned into the

vector and of thereby producing a polypeptide or protein. Expression of the cloned sequences occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Similarly, if a eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequences. Importantly, since eukaryotic DNA may contain intervening sequences, and since such sequences cannot be correctly processed in prokaryotic cells, it is preferable to employ cDNA from a cell which is capable of expressing c-SF-25 in order to produce a prokaryotic genomic expression vector library. Procedures for preparing cDNA and for producing a genomic library are disclosed by Maniatis *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982)).

The above-described expression vector genomic library is used to create a bank of host cells (each of which contains one member of the library). The expression vector may be introduced into the host cell by any of a variety of means (i.e., transformation, transfection, protoplast fusion, electroporation, etc.). The bank of expression vector-containing cells is clonally propagated, and its members are individually assayed (using an immunoassay) to determine whether they produce a protein capable of binding to SF-25 antigen.

The expression vectors of those cells which produce a protein capable of binding to SF-25 antigen are then further analyzed to determine whether they express (and thus contain) the entire c-SF-25 MAb gene, whether they express (and contain) only a fragment of the c-SF-25 MAb gene, or whether they express (and contain) a gene whose product, though immunologically related to c-SF-25 MAb, is not c-SF-25 MAb. Although such an analysis may be performed by any convenient means, it is preferable to determine the nucleotide sequence of the DNA or cDNA fragment which had been cloned into the expression vector. Such nucleotide sequences are then examined to

determine whether they are capable of encoding polypeptides having the same amino acid sequence as digestion fragments of SF-25.

An expression vector which contains a DNA or cDNA molecule which encodes the c-SF-25 MAb gene may, thus, be recognized by: (i) the ability to direct the expression of a protein which is capable of binding to SF-25 antigen; and (ii) the presence of a nucleotide sequence which is capable of encoding each of the fragments of c-SF-25 MAb. The cloned DNA molecule of such an expression vector may be removed from the expression vector and isolated in pure form.

Isolation of genomic DNA fragments containing the SF-25 light and heavy chain antibody variable region gene segments is also a method that may be used to clone the chimeric SF-25 MAb. In this method high molecular weight genomic DNA is isolated from the murine SF-25 cell line by standard techniques described in Maniatis *et al. supra*. This DNA can then be reduced to a smaller size by digestion with various restriction enzymes, by sonicating, by shearing by the application of mechanical force, or by a variety of other procedures. These genomic DNA fragments can then be ligated into various types of phage cloning vectors such as λgtWES, λgt10, or EMBL3 or plasmid cloning vectors such as pBR322, pUC18, or cosmid cloning vectors such as pHC79 and introduced into host bacterial. Other vectors and host organisms may be used to clone genomic SF-25 DNA fragments.

A collection of these genomic DNA fragments is referred to as a genomic DNA library. Once a library is constructed from the SF-25 genomic DNA, a variety of methods may be used to identify and/or isolate the genomic DNA fragments containing the SF-25 light and heavy chain antibody variable region gene segments. As described above, oligonucleotides capable of encoding a fragment of the SF-25 Mab gene may be labeled and used as a hybridization probe to isolate the genomic DNA fragment containing the SF-25 light or heavy chain variable region. Other nucleic acid probes that contain the mouse antibody constant region, the mouse J region, surrounding or flanking sequences, or sequences that corresponding to the SF-25 light and

heavy chain antibody variable region sequences such as a cDNA may be used as a hybridization probe.

It is contemplated that further "humanization" of the monoclonal antibodies of the invention may be accomplished by forming "mosaic" antibodies in which human sequences are also inserted into the variable region. For example, the variable regions of both mouse and human antibodies comprise four framework residues (FRs). Within the FRs are three complementarity determining residues (CDRs) which are responsible for antigen binding. A human-mouse mosaic having the desired binding characteristics may be made by inserting mouse CDR sequences within human framework residues. Such mosaic variants are contemplated equivalents of the chimeric immunoglobulins of the invention, as are partial chimeric immunoglobulins, e.g., in which only the heavy chain constant region of murine origin has been replaced by an equivalent sequence of human origin, or variants wherein one or more amino acids have been changed by directed mutagenesis.

3. Expression of the Cloned Chimeric SF-25 Gene

The present invention therefore provides a means for obtaining a DNA molecule which encodes the c-SF-25 MAb molecule. By operably linking this DNA molecule (or a fragment or mutated form of this DNA molecule) to a functional promoter, it is possible to direct the expression of the SF-25 MAb gene (or a fragment or derivative thereof) in a cell, or organism.

The expression of a DNA sequence requires that the DNA sequence be "operably linked" to DNA sequences which contain transcriptional and translational regulatory information. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, but shall in general include a promoter region which, in

prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal the initiation of protein synthesis. Regulatory regions in eukaryotic cells will in general include a promoter region sufficient to direct the initiation of RNA synthesis.

Two DNA sequences (such as a promoter region sequence and a c-SF-25 MAb-encoding sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of the c-SF-25 MAb-encoding sequence, or (3) interfere with the ability of the c-SF-25-MAb encoding sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of transcribing that DNA sequence.

To express the c-SF-25 MAb molecule (or a functional derivative thereof) in a prokaryotic cell (such as, for example, *E. coli*, *B. subtilis*, *Pseudomonas*, *Streptomyces*, etc.), it is necessary to operably link the c-SF-25 MAb-encoding sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ, the bla promoter of the β-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pPR325, etc. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the trp, recA, lacZ, lacI, and gal promoters of *E. coli* the α-amylase (Ulmanen *et al.*, *J. Bacteriol. 162*:176-182 (1985)) and the σ-28-specific promoters of B. subtilis (Gilman *et al.*, *Gene 32*:11-20 (1984)), the promoters of the bacteriophages of Bacillus (Gryczan In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward *et al.*, *Mol. Gen. Genet. 203*:468-478 (1986)). Prokaryotic promoters are reviewed by Glick, B.R., (*J. Ind. Microbiol. 1*:277-282 (1987)); Cenatiempo, Y.

(*Biochimie 68*:505-516 (1986)); and Gottesman, S. (*Ann. Rev. Genet. 18*:415-442 (1984)).

Proper expression in a prokaryotic cell requires the presence of a ribosome binding site upstream of the gene-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold *et al.* (*Ann. Rev. Microbiol. 35*:365-404 (1981)).

If expression is desired in a eukaryotic cell, such as yeast, fungi, mammalian cells, or plant cells, then it shall be necessary to employ a promoter capable of directing transcription in such a eukaryotic host. Preferred eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer *et al., J. Mol. Appl. Gen. 1*:273-288 (1982)); the TK promoter of Herpes virus (McKnight, S., *Cell 31*:355-365 (1982)); the SV40 early promoter (Benoist *et al., Nature (London) 290*:304-310 (1981)); and the yeast gal4 gene promoter (Johnston *et al., Proc. Natl. Acad. Sci. (USA) 79*:6971-6975 (1982); Silver *et al., Proc. Natl. Acad. Sci. (USA) 81*:5951-5955 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine of an oligonucleotide. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and an oligonucleotide which encodes the c-SF-25 MAb molecule (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the c-SF-25 MAb-encoding oligonucleotide) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the c-SF-25 MAb gene sequence).

An oligonucleotide which encodes the c-SF-25 MAb (or a functional derivative thereof) when operably linked to a functional promoter is preferably introduced into a recipient cell by any of a variety of suitable means: transformation, transfection, conjugation, protoplast fusion, electroporation, etc.

The c-SF-25 MAb-encoding sequence and an operably linked promoter may be introduced into a recipient cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the c-SF-25 MAb may occur through the transient expression of the introduced gene sequence. Alternatively, permanent expression may occur through the integration of the introduced gene sequence into the host chromosome.

Preferably, the introduced gene sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in E. coli (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX. Such plasmids are, for example, disclosed by Maniatis *et al.* (In: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982)). *Bacillus* plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, T. (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307-329). Suitable *Streptomyces* plasmids include pIJ101 (Kendall *et al., J. Bacteriol. 169*:4177-4183 (1987)), and streptomyces bacteriophages such as ϕC31 (Chater *et al.*, In: *Sixth International Symposium on Actinomycetales Biology*, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). *Pseudomonas* plasmids are reviewed by John *et al.* (*Rev. Infect. Dis. 8*:693-704 (1986)), and Izaki, K. (*Jpn. J. Bacteriol. 33*:729-742 (1978)).

Preferred eukaryotic plasmids include BPV, vaccinia, SV40, 2-micron circle, etc. Such plasmids are well known in the art (Botstein *et al., Miami*

Wntr. Symp. 19:265-274 (1982); Broach, J.R., In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, J.R., *Cell 28*:203-204 (1982); Bollon *et al., J. Clin. Hematol. Oncol. 10*:39-48 (1980); Maniatis, T., In: *Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Expression*, Academic Press, NY, pp. 563-608 (1980)).

A method of production of c-SF-25 may combine five elements:

1. Isolation of messenger RNA (mRNA) from the mouse hybridoma cell line which produces the SF-25 MAb, cloning and cDNA production therefrom;

2. Preparation of a full length cDNA library from purified mRNA from which the appropriate V region gene segments of the L and H chain genes can be: (i) identified with appropriate probes, (ii) sequenced, and (iii) made compatible with a C gene segment.

3. Preparation of C region gene segment modules by cDNA preparation and cloning.

4. Construction of complete H or L chain-coding sequences by linkage of the cloned specific immunoglobulin V region gene segments described in 2 above to cloned human C region gene segment modules described in 3.

5. Expression and production of chimeric L and H chains in selected hosts, including prokaryotic and eukaryotic cells.

One common feature of all immunoglobulin L and H chain genes and the encoded messenger RNAs is the so-called J region. H and L chain J regions (J_H and J_L) have different sequences, but a high degree of sequence homology exists (greater than 80%) among each group, especially near the C region. This homology is exploited in this invention wherein consensus sequences of J_H and J_L were used to design oligonucleotides for use as primers or probes for introducing useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments.

C region cDNA module vectors prepared from human cells and modified by site-directed mutagenesis to place a restriction site at the analogous position in the human sequence were used. For example, one can clone the complete human κ chain C region (C_κ) and the complete human gamma 1 C region ($C_\gamma 1$). An alternative method utilizing genomic C region clones as the source for C region module vectors would not allow these genes to be expressed in hosts such as bacteria where enzymes needed to remove intervening sequences are absent.

Cloned V region segments are excised and ligated to C_L or C_H module vectors. In addition, the human gamma 1 region can be modified by introducing a termination codon thereby generating a gene sequence which encodes the H chain portion of an Fab molecule.

The coding sequences with linked V and C regions are then transferred into appropriate expression vehicles for expression in appropriate hosts, prokaryotic or eukaryotic.

Two coding DNA sequences are said to be "operably linked" if the linkage results in a continuously translatable sequence without alteration or interruption of the triplet reading frame. A DNA coding sequence is operably linked to a gene expression element if the linkage results in the proper function of that gene expression element to result in expression of the coding sequence.

Expression vehicles include plasmids or other vectors. Preferred among these are vehicles carrying a functionally complete human C_H or C_L chain sequence having appropriate restriction sites engineered so that any V_H or V_L chain sequence with appropriate cohesive ends can be easily inserted. Human C_H or C_L chain sequence-containing vehicles are thus an important embodiment of the invention. These vehicles can be used as intermediates for the expression of any desired complete H or L chain in any appropriate host.

One preferred host is yeast. Yeast provides substantial advantages for the production of immunoglobulin L and H chains. Yeast carry out post-translational peptide modifications including glycosylation. A number of

recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for the production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e. prepeptides) (Hitzman *et al., 11th International Conference on Yeast, Genetics and Molecular Biology*, Montpellier, France, September 13-17, 1982).

Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of chimeric H and L chain proteins and assembled chimeric antibodies. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the iso-l-cytochrome C (CYC-1) gene can be utilized. A number of approaches may be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast.

Bacterial strains may also be utilized as transformation hosts for the production of antibody molecules or antibody fragments described by this invention. *E. coli* strains such as *E. coli* W3110 (ATCC 27325) and other enterobacteria such as *Salmonella typhimurium* or *Serratia marcescens*, and various *Pseudomonas* species may be used.

Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches may be taken for evaluating the expression plasmids for the production of chimeric antibodies or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria.

Other preferred hosts are mammalian cells, grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of H and L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein.

Mammalian cells which may be useful as hosts for the production of antibody proteins include cells of lymphoid origin, such as the hybridoma Sp2/O-Ag14 (ATCC CRL 1581) or the myeloma P3X63Ag8 (ATCC TIB 9), and their derivatives. Others include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO- K1 (ATCC CRL 61).

Many vector systems are available for the expression of cloned H chain and L chain genes in mammalian cells. Different approaches can be followed to obtain complete H_2L_2 antibodies. It is possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H_2L_2 antibodies. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both H and L chains can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells may be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with a H chain plasmid containing a second selectable marker. Cell lines producing H_2L_2 molecules via either route could be transfected with plasmids encoding additional copies of H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled (H_2L_2) antibody molecules or enhanced stability of the transfected cell lines.

B. Polypeptide Products

The present invention provides "chimeric" immunoglobulin chains, either H or L, with specificity toward human tumor cell SF-25 antigen. A

chimeric chain contains a C region substantially similar to that present in a natural human immunoglobulin, and a V region having the desired anti-tumor specificity of the invention.

The invention also provides immunoglobulin molecules having H and L chains associated so that the overall molecule exhibits the desired binding and recognition properties. Various types of immunoglobulin molecules are provided: monovalent, divalent, or molecules with the invention's V binding domains attached to moieties carrying desired functions.

This invention also provides for "fragments" of chimeric immunoglobulin molecules, which include Fab, Fab', and $F(ab')_2$ molecules. The invention also provides for "derivatives" of the chimeric immunoglobulins, which term includes those proteins encoded by truncated or modified genes to yield molecular species functionally resembling the immunoglobulin fragments. The modifications include, but are not limited to, addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins and tumor necrosis factor (TNF). The fragments and derivatives can be produced from any of the hosts of this invention.

Antibodies, fragments or derivatives having chimeric H chains and L chains of the same or different V region binding specificity, can be prepared by appropriate association of the individual polypeptide chains, as taught, for example by Sears *et al.* (*Proc. Natl. Acad. Sci. USA* 72:353-357 (1975)). With this approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

C. Diagnostic Uses of the Chimeric SF-25 Monoclonal Antibody

The c-SF-25 MAbs of the present invention are particularly suited for *in vivo* and *in vitro* imaging of certain tumors and other diseased tissues.

1. Labels

These chimeric antibodies, or fragments or derivatives thereof, may be labeled using any of a variety of labels and methods of labeling. Examples of types of labels which can be used in the present invention include, but are not limited to, enzyme labels, radioisotopic labels, non-radioactive isotopic labels, fluorescent labels, toxin labels, chemiluminescent labels, and nuclear magnetic resonance contrasting agents.

Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholine esterase, etc.

Examples of suitable radioisotopic labels include ^{3}H, ^{111}In, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{57}To, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, ^{90}Y, ^{67}Cu, ^{217}Ci, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd, etc. ^{111}In is a preferred isotope. Its use may have substantial advantages since its avoids the problem of dehalogenation of the ^{125}I or ^{131}I-labeled monoclonal antibody by the liver. In addition, this radionucleotide has a more favorable gamma emission energy for imaging (Perkins *et al., Eur. J. Nucl. Med. 10*:296-301 (1985); Carasquillo *et al., J. Nucl. Med. 28*:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhanced specificity of tumor localization (Esteban *et al., J. Nucl. Med. 28*:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Tr, ^{56}Fe, etc.

Examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a fluorescamine label, etc.

Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin, etc.

Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, Iron, etc.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to these chimeric antibodies, derivatives, or fragments thereof, can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy *et al.* (*Clin. Chim. Acta 70*:1-31 (1976)), and Schurs *et al.* (*Clin. Chim. Acta 81*:1-40 (1977)). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

2. *In vivo* Imaging Using the c-SF-25 MAb

The detection of cells which express the SF-25 antigen may be accomplished by the use of *in vivo* imaging techniques in which the labeled chimeric MAbs of the present invention, or fragments or derivatives thereof, are administered to a patient or other animal, and the presence of tumors or other diseased tissues expressing the antigen is detected without the prior removal of any tissue sample. Such *in vivo* detection procedures have the

advantage of being less invasive than other detection methods, and are, moreover, capable of detecting the presence of antigen-expressing cells in tissue which cannot be easily removed from the patient.

3. *In vitro* Imaging Using the c-SF-25 MAb

The chimerized antibodies or fragments or derivatives of the present invention are also particularly suited for use *in vitro* to detect cells which express the SF-25 antigen in body tissue, fluids (such as blood, lymph, etc.), stools, or cellular extracts.

The detection of cells which express the SF-25 antigen may be accomplished by removing a sample of tissue from a patient or other animal and then treating the isolated sample with any of the suitably labeled chimeric SF-25 antibodies of the present invention. Preferably, such *in vitro* detection is accomplished by removing a histological specimen from a patient or other animal, and providing the labeled chimeric antibodies of the present invention to such specimen by applying them or by overlaying them onto a sample of tissue. Through the use of such a procedure, it is possible to determine not only the presence of the SF-25 antigen, but also the distribution of the antigen on the examined tissue. Using the present invention, those of ordinary skill in the art will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in vitro* detection.

The c-SF-25 MAbs of the present invention may be used to quantitatively or qualitatively detect the presence of cells which express the SF-25 antigen. Thus the c-SF-25 MAb of the present invention may be employed to detect or visualize, by biopsy and histology, cancers or other diseased tissues which express the SF-25 antigen. Such detection may be accomplished using any of a variety of methods. For example, by radioactively labeling the cells of the present invention, it is possible to detect the targeted antigen through the use of radioimmune assays. A good

description of a radioimmune assay (RIA) may be found in *Laboratory Techniques and Biochemistry in Molecular Biology* by Work *et al.* (North Holland Publishing Company, NY (1978)), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein.

4. Use of c-SF-25 MAb in Immunoassays

The c-SF-25-MAbs, or fragments or derivatives thereof, of the present invention are particularly suited for use in immunodiagnostic assays for the SF-25 antigen wherein they may be utilized in a liquid phase or bound to a solid phase carrier and may be provided in kits.

These chimeric antibodies, or fragments or derivatives thereof, may be labeled using any of a variety of labels and methods of labeling. Examples of types of labels which can be used in the present invention include, but are not limited to, enzyme labels, radioisotopic labels, non-radioactive isotopic labels, fluorescent labels, toxin labels, and chemiluminescent labels, as described previously.

The detection of SF-25 antigens by the chimeric SF-25 antibodies, or fragments or derivatives thereof, of the present invention can be improved through the use of carriers. Well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to the SF-25 antigen. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Those skilled in the art will note many other suitable carriers for

binding monoclonal antibody, or will be able to ascertain the same by use of routine experimentation.

The chimeric SF-25 antibodies, or fragments or derivatives thereof, of the present invention may be used to quantitatively or qualitatively detect the presence of cells which express the SF-25 antigen. Such detection may be accomplished using any of a variety of immunoassays. For example, by radioactively labeling these chimeric derivatives or fragments thereof, it is possible to detect the SF-25 antigen through the use of radioimmune assays as previously described.

The c-SF-25 MAbs, derivatives or fragments thereof, of the present invention may also be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In such an immunometric assay, a quantity of unlabeled SF-25 MAbs or c-SF-25 MAbs is bound to a solid support that is insoluble in the fluid being tested (*i.e.*, blood, lymph, liquified stools, tissue homogenate, etc.) and a quantity of detectably labeled, c-SF-25 MAb or SF-25 MAbs is added to permit detection and/or quantitation of the ternary complex formed between solid-phase unlabeled antibody, the SF-25 antigen and the labeled antibody.

Typical immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the SF-25 antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted SF-25 antigen, if any, and then is contacted with the solution containing a known quantity of labeled c-SF-25 antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the SF-25 antigen bound to the solid support through the unlabeled antibody, the solid support is washed a second time to remove the unreacted labeled antibody. This type of forward sandwich assay may be a simple "yes/no" assay to determine whether SF-25 antigen is present or may be made quantitative by comparing the measure of labeled c-SF-25 antibody

with that obtained for a standard sample containing known quantities of SF-25 antigen. Such "two-site" or "sandwich" assays are described by Wide at pages 199-206 of *Radioimmune Assay Method*, edited by Kirkham and Hunter, E. & S. Livingstone, Edinburgh, 1970.

In another type of "sandwich" assay, which may also be useful with the antibodies of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the c-SF-25 antibody bound to the solid support and labeled c-SF-25 antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled c-SF-25 antibody. The presence of labeled c-SF-25 antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled c-SF-25 antibody to the fluid sample followed by the addition of unlabeled c-SF-25 antibody bound to a solid support after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the the residue of the sample being tested and the solution of unreacted, labeled c-SF-25 antibody. The determination of labeled c-SF-25 antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

As explained above, the immunometric assays for SF-25 antigen require that the particular binding molecule be labeled with a "reporter molecule." These reporter molecules or labels, as identified above, are conventional and well-known to the art. In the practice of the present invention, enzyme labels are a preferred embodiment. No single enzyme is ideal for use a label in every conceivable immunometric assay. Instead, one must determine which enzyme is suitable for a particular assay system. Criteria important for the choice of enzymes are turnover number of the pure enzyme (the numer of substrate molecules converted to produce per enzyme site per unit of time), purity of the enzyme preparation, sensitivity of detection

of its product, ease and speed of detection of the enzyme reaction, absence of interfering factors or of enzyme-like activity in the test fluid, stability of the enzyme and its conjugate, and the like. Included among the enzymes used as preferred labels in the immunometric assays of the present invention are peroxidase, alkaline phosphatase, beta-galactosidase, urease, glucose oxidase, glycoamylase, malate dehydrogenase, and glucose-6-phosphate dehydrogenase. Urease is among the more preferred enzyme labels, particularly because of chromogenic pH indicators which make its activity readily visible to the naked eye.

The above-described *in vitro* or *in vivo* detection methods may be used in the diagnosis of certain cancers, such as colon adenocarcinoma, or other cancers and diseases which express the SF-25 antigen. Additionally, such detection methods may be used to assist in the determination of the stage of a malignancy or other disease, or to determine whether an individual possesses malignant or other lesions which may be obscured (or whose detection may be complicated) by the close association of normal tissue.

One especially preferred use for the c-SF-25 MAbs of the present invention is as an aid in the diagnosis of colon cancer in patients who present with symptoms of inflammatory bowel diseases, and in particular, ulcerative colitis or intestinal polyps. Using the methods of the prior art, the early diagnosis and detection of colon cancer in individuals suffering from such inflammatory bowel disease is often complicated, or masked, by the symptoms of bowel disease. Thus, concern that an occult colorectal carcinoma may be present in an individual suffering from inflammatory bowel disease may result in a recommendation that such individuals submit to a colectomy. Because the c-SF-25 MAbs of the present invention are capable of identifying colorectal carcinomas, they can be used to determine the presence of otherwise occult lesions. Thus, their use in the diagnosis of the cause and severity of inflammatory bowel disease and colorectal carcinoma is capable of preventing unwarranted colectomies, and is, therefore, highly desirable.

As used herein, an effective amount of c-SF-25 MAbs is one capable of achieving the desired diagnostic discrimination. The amount of c-SF-25 MAb which is typically used in a diagnostic test is generally between 1-10 mg, and preferably between 1μg to 1mg.

D. Therapeutic Uses of the Present Invention

In addition to providing methods for diagnosing and treating colon cancer, pancreatic andenocarcinoma, or other cancers of endodermal origin, the c-SF-25 MAbs of the present invention also provide a means for preventing the onset of these cancers, and for treating affected animals including humans. The discovery that the SF-25 antigen is constitutively expressed on colon cancer cells, and the identification of c-SF-25 MAbs capable of binding to this antigen provide a means for preventing and treating these cancers and other diseased tissues which express the SF-25 antigen.

The antibodies of this invention can be adapted for therapeutic efficacy by virtue of their ability to mediate ADCC and/or complement-dependent cytotoxicity (CDC) against tumor cells. For these activities, either an endogenous source or an exogenous source of effector cells (for ADCC) or complement components (for CDC) can be utilized.

The chimeric antibodies, fragments or derivatives of this invention may be advantageously utilized in combination with other chimeric antibodies, or with lymphokines or hemopoietic growth factors, etc., which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention having a complete or partial human C region can be introduced *in vivo*, especially in humans, with reduced negative immune reactions such as serum sickness or anaphylactic shock, as compared to whole mouse antibodies.

The ability to conjugate the chimeric SF-25 antibodies, or fragments or derivatives thereof, with toxin molecules provides an additional method for treating colon cancer and other cancers and diseased tissues which

constitutively express the SF-25 antigen. In this embodiment, the c-SF-25 MAb, or derivatives or fragments of these antibodies, which are capable of recognizing the SF-25 antigen, are conjugated with toxin molecules and administered to a patient suspected of having colon or other cancer. When such a toxin derivatized MAb binds to a colon or cancer cell, the toxin moiety will cause the death of the cancer cell.

Any of a variety of toxin molecules may be employed to produce such toxin-conjugated, c-SF-25 MAbs. Examples of suitable cytotoxic molecules include: ricin; diphtheria, pseudomonas, and cholera toxins; TNF, etc. Toxins conjugated to antibodies or other ligands are known in the art (see, for example, Olsnes *et al., Immunol. Today 10*:291-295 (1989)).

Additional types of therapeutic moieties including, but not limited to, radionuclides and cytotoxic drugs and other agents, can be conjugated to the c-SF-25 MAbs of the present invention to treat cancer patients. Examples of radionuclides which can be coupled to the cells of the present invention and delivered *in vivo* to sites of the SF-25 antigen include ^{212}Bi, ^{131}I, ^{125}O, ^{186}Re, and ^{90}Y, which list is not intended to be exhaustive. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy.

Cytotoxic drugs which can be conjugated to the c-SF-25 MAbs of the present invention and subsequently used for *in vivo* therapy include, but are not limited to: alkylating agents such as nitrogen mustards (e.g., cyclophosphamide), ethylenimines (e.g., thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., lomustine and semustine) and triazenes (e.g., dacarbazine); antimetabolites such as folic acid analogs (e.g., methotrexate), pyrimidine analogs (e.g., 5-fluorouracil and fluorodeoxyuridine), and purine analogs and related inhibitors (e.g., 6-mercaptopurine, and pentostatin); natural products such as vinca alkaloids (e.g., vinblastine and vincristine), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, and mitomycin C), enzymes (e.g., L-asparaginase), and biological response modifiers (e.g., interferon alpha); miscellaneous agents

such as platinum coordination complexes (e.g., cisplatin), anthracenediones (e.g., mitoxantrone), substituted ureas (e.g., hydroxyurea), methy hydrazine derivatives (e.g., procarbazine), adrenocortical suppressants (e.g., aminoglutethimide); and hormones and antagonists, such as adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone), estrogens (e.g., ethinyl estradiol), antiestrogens (e.g., tamoxifen), androgens (e.g., fluoxymesterone), antiandrogens (e.g., flutamide) and/or gonadotropin-releasing hormone analogs (e.g., leuprolide). Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and protein synthesis. For a fuller exposition of these classes of drugs which are known in the art, and their mechanisms of action, see Calabresi, P., *et al.*, Chemotherapy of Neoplastic Diseases (pp. 1202-1208) and Antineoplastic Agents (pp. 1209-1263), both in *Goodman and Gilman's THE PHARMACOLOGICAL BASIS OF THERAPEUTICS* (Gilman, A.G., *et al.*, eds.), 8th Edition, Pergamon Press, New York, NY (1990).

One preferred type of toxin molecule is a "photo-activatable toxin molecule." Examples of such "photo-activatable toxin molecules" include Photofrin II (Williams et al., *Photochem. Photobiol. 46*:733-738 (1987); Mattielli et al., *Photochem. Photobiol. 46*:873-880 (1987)), hematoporphyrin derivatives (Benson, R.C., *Urology 31*:13-17 (1988)), hemoglobin, and its derivatives (Polla et al., *Ann. Dermatol. Venereol. 114*:497-505 (1987)); procion blue (Macklis et al., *Brain Res. 359*:158-165 (1985)), fluorescene, and other dyes (Miller et al., *Science 206*:702-704 (1979); Manyak et al., *J. Clin. Oncol. 6*:380-391 (1988)), etc. The critical attribute of such molecules is that they are capable of greater absorption of light (at some wavelength) than the surrounding tissue.

In this therapy, termed "photothermolysis," photo-activation of the toxin is achieved by a careful selection of wavelength, pulse, and intensity of the light. The light energy absorbed by such molecules is released either as heat or emitted as light at a different wavelength. If a suitable light (such as, preferably, a laser light) is employed, the death of cells and tissue which

contain the photo-activatable toxin will occur, either because of the amount of the heat released by this process, or because of the photo-oxidation of essential biological molecules in the cells or tissue by the emitted light. The physics of laser therapy and photothermolysis are reviewed by Hobbs et al. (*J. Dermatolog. Surg. Oncol. 13*:955-964 (1987)), Anderson *et al.* (*Science 220*:524-527 (1983)), Macklis *et al.* (*Brain Res. 359*:158-165 (1985)), Wilson, B.C. (*Phys. Med. Biol. 31*:327-360 (1986)), and especially by Manyak *et al.* (*J. Clin. Oncol. 6*:380-391 (1988)), all of which are incorporated herein by reference.

By conjugating photo-activatable toxins with the chimeric MAbs of the present invention, it is possible to direct the toxin molecule only to those cells which express the SF-25 antigen. Conjugating photo-activatable toxins to antibodies has been used to provide a selective means for treating a tumor without damage to normal (i.e. non-antigen expressing) cells. Examples of the use of this method are provided by: Mew *et al. (Cancer Res. 45*:4380-4386 (1985); *J. Immunol. 130*:1473-1477 (1983)); by Wat *et al., Prog. Clin. Biol. Res. 170*:351-360, (Doiron *et al.* eds.), Alan R. Liss, NY (1984)); and by Oseroff *et al. (Photochem. Photobiol. 46*:83-96 (1987)); *Id. 43 Suppl.*:105s (1986); *Id. 41 Suppl.*:75s (1985); *Id. 41 Suppl.*:35s (1985); *Proc. Natl. Acad. Sci. (USA) 83*:8744-8748 (1986); *Clin Res. 33*:674a (1985); *J. Invest. Dermatolog. 84*:335 (1985)); all of which references are herein incorporated by reference.

The above-described photothermolysis therapy can be accomplished using any light source which is capable of photo-activating the toxin. The photo-activation of such toxins can thus be achieved using light sources other than lasers. For example, such photo-activation can be achieved using light from an ordinary light bulb (Dougherty *et al., J. Natl. Canc. Inst. 55*:115-129 (1979); Wilson, B.C., *Phys. Med. Biol. 31*:327-360 (1986)). Photo-activation of the toxin may alternatively be achieved by the administration of a chemiluminescent agent (i.e. a light-emitting molecule) to an individual who has recieved the photo-activatable toxin. This embodiment of the present

invention is particularly advantageous in the *in situ* treatment of gastric carcinomas, intestinal polyps, and Barrett's esophagus. This embodiment may also be used for metastatic cancers (Phillip *et al.*, In: Porphyrin Localization and Treatment of Tumors (Doiron *et al.*, eds.), Alan R. Liss, NY, pp. 563-569 (1985)).

The therapeutic compositions which contain the c-SF-25 MAbs of the present invention, and/or their conjugates just described, can be administered orally or parenterally by the intravenous, intramuscular, subcutaneous, rectal, transdermal, intrapulmonary, intraperitoneal, intrathecal, intratumoral, intranasalpharyngeal or other known routes of administration. These therapeutic compositions will be manufactured by methods which are well known to those of skill in the art.

As would be understood by one of ordinary skill in the art, such therapeutic compositions may contain salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the c-SF-25 MAb and its conjugates.

Compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Examples of non-aqueous solvents are propylene glycol, polyethylene gylcol, vegetable oils, such as olive oil, and injectable organic esters such as ethyl oleate.

Carriers or occlusive dressings can be used to increase skin permability and enhance antibody absoprtion.

Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water.

Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents.

Adjuvants are substances that can be used to specifically augment a specific immune response. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the animal being immunized. Adjuvants can be loosely divided into several groups based upon their composition. These groups include oil adjuvants (for example, Freund's complete and incomplete), mineral salts (for example, $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, silica, kaolin, and carbon), polynucleotides (for example, poly IC and poly AU acids), and certain natural substances (for example, wax D from *Mycobacterium tuberculosis*, as well as substances found in *Corynebacterium parvum*, or *Bordetella pertussis*, and members of the genus *Brucella*). Among those substances particularly useful as adjuvants are the saponins such as, for example, Quil A. (Superfos A/S, Denmark). Examples of materials suitable for use in vaccine compositions are provided in *Remington's Pharmaceutical Sciences* (Osol, A., Ed., Mack Publishing Co., Easton, PA, pp. 1324-1341 (1980)).

Treatment of an individual with a tumor bearing the SF-25 antigen recognized by the c-SF-25 MAbs of this invention comprises admininstering an effective amount of this c-SF-25 MAb, or fragments or derivatives thereof in a single dose, multiple doses or an infusion of these chimeric MAbs and/or their conjugates to a patient or other animal.

According to the present invention, an "effective amount" of a therapeutic composition is one which is sufficient to achieve the desired biological effect. Generally, the dosage needed to provide an effective amount of the composition, and which can be adjusted by one of ordinary skill in the art, will vary depending upon such factors as the individual chimeric antibody used, the presence and nature of any therapeutic agent conjugated thereto, the

animal's or patients age, condition, sex, and clinical status including extent of disease, if any, and other variables.

The effective dosage can vary from about 10 ng/kg body weight to about 100 mg/kg body weight. Effective concentrations of the compositions of the invention can vary from 0.01-1,000 μg/ml per dose.

E. The SCID Mouse Model

Since hepatic metastases are often a complication of colorectal cancer, a SCID mouse model was established using LS 180 human colon adenocarcinoma cells to evaluate the potential anti-tumor effects of chimeric SF-25. In an attempt to provide a control for the human IgG1 Fc component of this chimeric MAb in functional studies, the results were compared to other chimeric MAbs.

The SCID mouse model is useful because: 1) the blood supply to the tumor cells in the liver is substantially better than that to tumor cells grown in previously described models that used subcutaneous tumor xenografts; and 2) the SCID mouse lacks both T and B cells and therefore will accept xenografts of normal and tumor human tissues (*Proc. Curr. Top. Microbiol. Immunol. 152*:1-263 (1989); Bosma *et al., Ann. Rev. Immunol. 9*:323-350 (1991); McCune *et al., Science 241*:1632-1639 (1988); Mosier *et al., Nature 335*:256-259 (1988)).

Applicants have produced a mouse-human c-SF-25 MAb and demonstrated that: the c-SF-25 MAb selectively binds to the SF-25 antigen; the c-SF-25 MAb induced ADCC by human PBLs of LS 180 human-derived tumor cells; the c-SF-25 MAb inhibited the development of hepatic metastases of human colon adenocarcinoma cells; mice treated with c-SF-25 MAb had a statistically significant increased survival rate compared to control mice injected with LS 180 cells; and that the stable binding of c-SF-25 MAb to the tumor cell surface by its antigen binding site at physiological temperature (37°C) is important for its anti-tumor effect.

Having now generally described the invention, the same will be more readily understood through reference to the following methods and examples, which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1

Preparation of Chimeric SF-25 MAb

Cell Lines

The murine hybridoma cell line SF-25 secretes a monoclonal antibody of IgG1 heavy chain and Kappa light chain isotype that binds to a 125 kD cell surface antigen called the SF-25 antigen which is found on a variety of human adenocarcinoma cell lines of endodermal origin. It was obtained by fusing SP2/0-AG14 cells with spleen cells from a Balb/c mouse which had been immunized with the human hepatocellular carcinoma cell line FOCUS.

SP2/0-AG14 (SP2/0), and SF-25 cells were grown in Iscove's modified Dulbecco's medium (Hazleton Biologics, Inc., Lenexa, KS) supplemented with 5% FCS (Cell Culture Laboratories, Cleveland, OH) and 2 mM L-glutamine (Hazleton).

Purification of the Chimeric SF-25 Antibody

Cell culture supernatant was adjusted to 2 mM EDTA, 140 mM NaCl, and 20 mM Tris pH 8.5 and applied to a protein A sepharose column (Pharmacia, Piscataway, NJ). Chimeric SF-25 IgG1 antibodies was eluted with 0.1 M sodium citrate pH 3.5, neutralized with 1M Tris base and dialyzed against PBS.

Cloning of the SF-25 Variable Region Genes

High molecular weight DNA and cytoplasmic RNA was isolated from SF-25 cells by standard procedures. For Southern blot analysis, genomic DNA was digested with restriction endonucleases (New England Biolabs, Beverly, MA), fractionated by electrophoresis through a 0.8% agarose gel, and transferred to MagnaGraph (MSI, Westborough, MA). dCTP ^{32}P-labeled probes were prepared using a random priming labeling kit (Boehringer Mannheim, Indianapolis, IN).

To clone the heavy chain variable region, 150 μg of SF-25 DNA was digested with EcoR1 and size fractionated on a preparative 0.8% agarose gel. DNA fragments 4-5 kb in size were excised, electroeluted, ligated to EcoR1 digested lambda gt10 (Promega Biotech, Madison, WI) and packaged *in vitro* (Stratagene, LaJolla, CA). Twenty thousand plaques per 150 MM plate were screened using a J_H probe specific for the J segment of the murine heavy chain locus. To clone the light chain variable region, 150 μg of SF-25 DNA was digested with HindIII and size-fractionated on a preparative 0.8% agarose gel. DNA fragments 3-4 kb in size were excised, electroeluted, ligated to HindIII-digested Charon 27 and packaged *in vitro*. The phages were screened as above using a J_K probe specific for the murine J region of the kappa locus. Hybridizations were in 5X SSC (1X SSC = 0.15 M NaCl/0.015 M sodium citrate), 2X Denhardt's (1X Denhardt's = 0.02% each Ficoll 400, polyvinyl pyrrolidine and bovine serum albumin), 0.1% SDS, 200 μg/ml salmon sperm DNA, and 50% (vol/vol) formamide. Final washes were in 0.5X SSC at 65°C. Positive clones were plaque purified by three rounds of screening.

Generation of Chimeric Antibody-Producing Cell Lines

Vector pSF25kapgpt (Figure 2A) and pSF25 G1 apgpt (Figure 2B) were linearized with BamHI and transfected into the non-producing mouse myeloma cell line SP2/0 by electroporation using a BioRad Gene Pulser (Bio-

Rad Laboratories, Richmond, CA). $2x10^7$ cells were suspended in 0.8 ml Hanks buffered saline solution with 10 μg of each expression plasmid, previously digested with BamHI, and subjected to a pulse of 200 volts.

Cells were placed on ice for 10 minutes and plated at a density of 1 x 10^5 cells/ml in 96 well microtiter dishes. Selection was applied 48 hours later (0.5 μgml mycophenolic acid, 2.5 μg/ml hypoxanthine, 50 μg/ml xanthine; Sigma Chemical Company, St. Louis, MO) and resistant colonies were expanded into stable cell lines.

Clones were assayed for antibody production by an Elisa assay using goat-anti-human IgG (Fc Fragment specific) coating antibody and goat anti-human IgG (H+L) alkaline phosphatase conjugated antibody (Jackson ImmunoResearch, West Grove, PA). Standard curves were generated using chimeric antibodies purified by protein A Sepharose chromatography. The highest producing cell lines were subcloned by limiting dilution and evaluated for antibody production in T75 flasks. Growth curves were obtained by seeding cultures at 1 x 10^5 cells/ml in triplicate T75 flasks and taking daily samples for cell counts and ELISA assays. Chimeric MAb producing cell lines were grown under mycophenolic acid selection (see above) in Iscove's media plus 5% FCS.

Cloning of the SF-25 Light and Heavy Chain Genes

Southern blot analysis of SF-25 DNA was first performed using light and heavy chain specific J region probes. These DNA probes (Figure 1) hybridize to antibody variable region genes that have rearranged to the J locus as well as germline (unrearranged) DNA segments. We compared the hybridization patterns of SF-25 DNA with that seen in DNA from the fusion partner SP2/0. A unique 3.2 kb HindIII fragment in SF-25 hybridized to the light chain probe while a unique 4.5 kb Eco R1 fragment in SF-25 hybridized to the heavy chain probe. These DNA fragments were not in the fusion

partner SP2/0 and most likely represented the functionally rearranged variable region genes.

HindIII or EcoR1 DNA fragments containing the above SF-25 hybridizing fragments were purified from preparative agarose gels, ligated to either HindIII digested Charon 27 (light chain library) or Eco R1-digested λgt10 (heavy chain library), and packaged *in vitro*. Phage clones hybridizing to the immunoglobulin light and heavy chain probes were plaque purified and characterized by restriction enzyme analysis.

A 3.2 HindIII fragment hybridizing to the light chain probe was isolated from the Charon 27 library. Restriction maps of the mouse light chain K region, the light chain probe and the SF-25 light chain 3.2 HindIII fragment are shown in Figure 1A. This 3.2 HindIII fragment was subcloned into the human Kappa light chain expression vector pHuKapgpt, which confers resistance to mycophenolic acid when transfected into mammalian cells.

A 4.5 kb EcoR1 fragment hybridizing to the heavy chain probe was isolated from the λgt10 library. Restriction maps of the mouse heavy chain J region, the mouse heavy chain probe used for hybridization and the SF-25 heavy chain 4.5 Kb EcoR1 DNA fragment are shown in Figure 1B. This 4.5 Kb EcoR1 fragment was subcloned into the human IgG1 heavy chain vector. Both light and heavy chain fragments hybridized to the appropriately sized RNA from cell line SF-25 indicating that they were expressed. The above method of cloning genomic DNA fragments utilizes the endogenous promoters of the SF-25 light and heavy chain genes and maintains the original exon and intron arrangements of each gene.

Expression of Chimeric SF-25 MAb in SP2/0 Cells.

The SF-25 light and heavy chain expression vectors were linearized at the BamHI site and electroporated into the mouse myeloma cell line SP2/0. Transfectants producing antibody were expanded from 96 well plates and were

evaluated for antibody production and stability in T75 flasks over several passages.

Chimeric GA733 (Ross *et al., Biochem. Biophys. Res. Commun. 135*:297-303 (1986)), 323/A3 (Edwards *et al., Cancer Res. 46*:1306-1317 (1986)) were prepared.

EXAMPLE 2

Cell Lines

The FOCUS cell line was developoed in Dr. Wand's laboratory (Lun *et al., In Vitro (Rockville) 20*:493-504 (1984)). All other cell lines were obtained from the American Type Culture Collection (Rockville, MD). All cell lines were maintained in Eagle's MEM (M.A. Bioproducts, Walkersville, MD) supplemented with 10% fetal calf serum inactivated at 56°C for 30 minutes, 10 μM nonessential amino acids, 100 U/ml penicillin, and 100 μg/ml streptomycin. In some experiments cells were maintained in RPMI 1640 (Hazleton Biologics, Inc., St. Lenexa, KS) supplemented with 10% heat-inactivated fetal calf serum, 10 μM nonessential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Cells used for *in vitro* testing were harvested from the monolayer cultures by treatment with 0.04% EDTA/versine buffer in the absence of trypsin for 5 minutes at 37°C. For production of xenografts in nude mice, a human colonic carcinoma cell line, LS 180, was harvested from tissue culture by overlaying subconfluent monolayer cultures with 0.05% trypsin and 0.02% EDTA solution in HBSS (GIBCO Laboratories, Grand Island, NY) for 5 minutes and injected as described below. All the cell lines used were demonstrated to be free of mycoplasma contamination by a nucleic acid hybridization technique using Mycoplasma T.C. 11 (Gen-Probe Inc., San Diego, CA).

Purification and Radiolabeling of Antibodies

The monoclonal antibodies were purified by Sepharose CL-4B Staphylococcal Protein A-affinity column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) and labeled with ^{125}I using the Iodogen method (Fraker *et al.*, *Biochem. Biophys. Res. Commun.* *80*:849-857 (1978)). Specific activities of iodinated MAbs were calculated as a ratio of cpm of radionuclide attached per mole of antibody (cpm/mol).

Association Constants

A constant number of LS 180 cells (10^5/well) was incubated with 100 μl of ^{125}I-labeled SF-25 MAb serially diluted with 20% bovine serum/PBS for 4 hours at 4°C or 37°C using 96-well filter-bottomed plates (V & P Scientific Inc., San Diego, CA; Takahashi *et al.*, *Cancer Res.* *48*:6573-6579 (1988)). Cells were then washed three times with PBS to remove unbound antibody and bound radioactivity was counted in the gamma well counter. The concentration of MAbs and the amount of bound MAbs were calculated from radioactivity (cpm) and specific activity (cpm/mol) of ^{125}I-labeled MAbs. The association constant (K_A) of MAb and the number of antibody binding sites per tumor cell (Bmax) were determined by the methods and equation described by Frankel and Gerhard (*Molec. Immunol.* *16*:191-106 (1979)).

Animals

Four to five week old male athymic Balb/c nude mice were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana). Throughout the experiments, these animals were maintained under specific-pathogen-free-conditions.

Isolation of Human Macrophage and NK-Cells

Venous blood was drawn from healthy volunteers with heparin (200 U/ml in final concentration) and overlain on Ficoll-Paque (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). Mononuclear cells were isolated by centrifuging at 1,200 r.p.m. for 30 minutes at 25°C and were washed three times with RPMI 1640 medium.

Mononuclear cells, adjusted to 5 x 10^6/ml, were incubated on a plastic plate for two hours at 37°C in a CO_2 incubator and macrophages were collected from adherent cells. The purity of the macrophage population was examined by flow cytometric analysis using anti-human monocyte/macrophage MAb, anti-Leu-M3 labeled with FITC (Dimitriu-Bona *et al., J. Immunol. 130*:145-152 (1983); Becton Dickinson Immunocytometry Sys., Mountain View, CA) as described below.

Human NK-cells were isolated by discontinuous density gradient centrifugation as large granular lymphocytes (LGLs) (Timonen *et al., J. Immunol. Method 36*:285-291 (1980)). Non-adherent cells, adjusted to 1 X 10^8 cells/gradient, were separated into fractions by centrifugation at 300 X g for 1 hour at 20°C on a six-step discontinuous density gradient of Percoll (Pharmacia LKB Biotech., Inc., Piscataway, NJ) at concentrations of 37.5, 40.0, 42.5, 45.0, 47.5, and 50% (osmolarity adjusted to 290 mOsm/kg by 10 X PBS). LGLs were collected from the 40.0% concentra-tion of Percoll. The purity of human NK-cells was examined by flow cytometric analysis using FITC labeled anti-Leu-11a, anti-human NK-cell antigen associated with IgG Fc receptor III (FcγRIII, CD16) (Phillips *et al., J. Exp. Med. 159*:993-1008 (1984)) as described below.

Isolation of Murine Peripheral Blood Lymphocytes

Blood was collected from the axillary artery and vein and was transferred to 15 ml Falcon 2099 conical plastic tubes filled with 10 ml of ACT solution (a mixture of 9 volumes of 0.83% NH_4Cl and 1 volume of 0.17M Tris HCl, pH 7.67) containing 200 U of heparin to hemolyze red blood cells. These peripheral blood leukocytes were washed with PBS and were resuspended in 30% metrizamide (Sigma Chemical Co., St. Louis, MO) in HBSS at 4°C at a final ratio of 7 parts metrizamide to 5 parts of packed blood cells in order to isolate lymphocytes. The mixture was transferred into 15 ml conical tubes, overlain with PBS and centrifuged at 1,400 X g for 20 minutes at 4°C. The lymphocyte layer was then carefully removed from the metrizamide-PBS interface and washed three times with PBS.

Preparation of Peritoneal Exudate Cells and Thioglycolate-Elicited Macrophages

Mice were injected intraperitoneally with 5 ml of 0.01 M PBS, pH 7.2. A few minutes later peritoneal exudate cells were obtained by aspirating PBS from the peritoneum cavity. The purity of macrophages was tested by flow cytometry using F4/80 anti-murine macrophage MAb as described below (Austyn *et al., Eur. J. Immunol. 11*:805-815 (1981)).

In order to obtain thioglycolate-elicited macrophages, nude mice were inoculated intraperitoneally with 1 ml of thioglycolate medium (Difco; VWR Scientific Corp., Philadelphia, PA). Four days later, peritoneal exudate cells were collected and were used as thioglycolate-elicited macrophages.

Preparation of Mouse Splenocytes

Mice were anesthetized with ether and were sacrificed by axillary bleeding. The peritoneal cavity was exposed under sterile conditions and the spleen was excised into a plastic dish containing 5 ml of RPMI 1640 medium.

Next, a small incision was made in the spleen and splenocytes were isolated by squeezing repeatedly with forceps. Splenocytes were then transferred into a plastic tube and were washed twice with RPMI 1640 medium.

Isolation of Murine NK-Cells

Murine NK-cells were isolated by discontinuous density gradient centrifugation as a LGL fraction (Lafreniere *et al., Cancer Res. 50*:1658-1666 (1990)). Non-adherent cells were obtained from murine splenocytes after incubation in a plastic dish for 2 hours at 37°C and were adjusted to 5 x 10^7 cells/gradient. They were separated into fractions by centrifugation at 300 X g for 1 hour at 20°C on a seven-step discontinuous density gradient of Percoll at concentrations of 20, 40, 50, 60, 70, and 100%. (The osmolarity was adjusted to 320 mOsm/kg by 10 X PBS). LGLs were collected from the interfaces of 20/40% and 40/50% concentrations of Percoll. The purity of murine NK-cells were examined by flow cytometric analysis using rabbit antiserum to ganglio-N-tetraosylceramide (anti-asialo GM1; Wako Chemicals, Dallas, TX; Kasai *et al., Eur. J. Immunol. 10*:175-180 (1980)) as described below.

Isolation of Lymphocytes from Mouse Liver

Lymphocytes were isolated from murine liver by using the method of Wiltrout *et al. (J. Exp. Med. 160*:1431-1449 (1984)), with some modifications. Mice were anesthetized with ether and sacrificed by axillary bleeding. The peritoneal cavity was exposed under sterile conditions and the portal vein was cannulated with a 27-gauge needle. Five ml of RPMI 1640 containing 10% FCS and antibiotics were injected to flush blood out of the liver through the portal vein. The liver was then excised and the gall bladder was removed. Next, the liver was minced into small pieces with a razor blade and incubated with 10 ml of prewarmed Mg++ free HBSS containing 5%

FCS, Pc 100 IU/ml, SM 100 μg/ml, 5 mM $CaCl_2$, 0.05% collagenase type IV, and 0.002% DNAse I type II (Sigma Chemical Co. St. Louis, MO) for 30 minutes at 37°C with shaking. The liver digest was then filtered through a 50-gauge stainless steel mesh using a sterile syringe plunger, and was washed twice with HBSS. The packed liver digest was then resuspended in 30% metrizamide in HBSS at 4°C at a final ratio of 7 parts metrizamide to 5 parts of packed liver digest. Three ml of the mixture were transferred into 15-ml conical tubes, overlain with 1.5 ml of PBS and centrifuged at 1,400 X g for 30 minutes at 4°C. The lymphocyte layer was then carefully removed from the metrizamide-PBS interface and washed three times with PBS.

Detection of Human Macrophages and NK-Cells by Flow Cytometry

Fifty μl of cell suspension adjusted to 2 x 10^7 viable cells/ml in 0.01 M PBS, pH 7.2 containing 1% BSA and 20 μl of FITC labeled anti-Leu-M3 (anti-human monocyte/macrophage MAb) or anti-Leu-11a (anti-human NK MAb) were incubated for 1 hour at 4°C. After washing three times with PBS, fluorescence was detected in a FACScan (Beckton Dickinson, Mountain View, CA.) for single color flow cytometric analysis. Results are expressed as mean percent of positive staining cells and mean linear fluorescence.

Flow Cytometric Analysis

Murine cells (1 x 10^6) were incubated with 200 μl of rat anti-mouse macrophage MAb F4/80 producing hybridoma culture supernatant (Austyn *et al., Eur. J. Immunol. 11*:805-815 (1981)) for 1 hour at 4°C in a Falcon 2054 plastic tube. After washing three times with cold PBS, the cells were incubated at 4°C for 1 hour with 200 μl of biotin-conjugated anti-rat IgG antibody made in rabbits (Vector Laboratory, Burlingame, CA) adjusted to 7.5 μg/ml in PBS containing 1% BSA. The cells were washed three times with cold PBS, were incubated for another 1 hour at 4°C with 200 μl of avidin

FITC (Becton Dickinson, Mountain View, CA), and were diluted 1:25 with PBS containing 1% BSA. After washing three times with PBS, fluorescence was detected in a Becton Dickinson FACScan. In some experiments macrophages were also examined by non-specific esterase staining.

Alternatively, 1 X 10^6 LS 180 cells were incubated for 1 hour at 4°C with 200 μl of murine SF-25 MAb adjusted to 1 mg/ml in 0.01 M PBS, pH 7.2, containing 1% bovine serum albumin. After washing three times at 4°C, the cells were further incubated at 4°C or 37°C for 15 minutes to 2 hours, and then were reacted for 1 hour at 4°C with fluorescein-conjugated goat anti-mouse IgG (Cappel Lab., Cochranville, PA), diluted 1:100 with 1% bovine serum albumin in PBS. Fluorescence was detected in a Becton Dickinson FACScan for single color flow cytometric analysis. Results are expressed as mean percent of positive staining cells.

Detection of Murine NK Cells by Flow Cytometry

Anti-asialo GM1 rabbit serum was used to detect the NK cells in nude mice by flow cytometry. The cells were collected and adjusted to 5 x 10^6 viable cells/ml in PBS containing 1% BSA. Two hundred μl of this cell suspension and 20 μl of anti-asialo GM1, adjusted to 5 μg/ml, were incubated for 1 hour at 4°C. The cells were washed three times with cold PBS, incubated with 200 μl of FITC-labeled $F(ab')_2$ fragments of goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO), diluted 1:40 with PBS containing 1% BSA were incubated for another one hour at 4°C. Fluorescein bound MAb was detected in a Becton Dickinson FACScan for single color flow cytometric analysis.

ADCC Assay

LS 180 cells were used as the target cells. Confluent cells were harvested with EDTA/Versene buffer and radiolabeled by incubating 1 x 10^6 cells with 100 μCi of sodium chromate-^{51}Cr (New England Nuclear, Boston, MA) for 30 minutes at 37°C. After washing, the radiolabeled cells were adjusted to 1 x 10^5/ml of RPMI 1640. One hundred μl of target cells and 50 μl of MAb (10 μg/ml in RPMI 1640) were pipetted into 96-well U bottom plates and then 100 μl of various concentrations of human PBL (1 x 10^6 - 1 x 10^7 lymphocytes/ml) were added to each well as effector cells. The final concentration of MAb in ADCC was adjusted to 20 μg/ml, since this concentration had been shown to be optimal in preliminary experiments. Plates were incubated in a CO_2 incubator at 37°C for 4 hours when human cells were used as effector cells, and for 8 hours when murine effector cells were used. After centrifugation of the U bottom plates at 1,500 rpm for 15 minutes, 200 μl of culture supernatant was collected and radioactivity was determined by a gamma well counter.

The spontaneous release of ^{51}Cr was measured after the incubation of target cells alone with RPMI 1640 and the total count was determined after the incubation of the cells in 1.0 N HCl. The spontaneous release was less than 10% of the total release in all experiments. The percent specific lysis was determined by the following formula:

$$\frac{\text{Observed cpm - spontaneous cpm}}{\text{Total cpm-spontaneous cpm.}} \times 100 = \% \text{ specific lysis.}$$

Animal Model for Liver Metastases of Human Colon Adenocarcinoma

Nude mice were anesthetized with 0.4 ml of 2% chloral hydrate (Sigma Chemical Co., St. Louis, MO) by ip. injection and placed in the decubitus position. A transverse incision was made in the left flank through the skin and peritoneum, exposing the spleen. Mice were injected with 1.0 x 10^6 LS 180 cells and 20 μg of rabbit anti-serum to ganglio-N-tetraosylceramide (anti-asialo GM1; Wako Chemicals, Dallas, TX) in 0.25 ml PBS into the portal vein via the splenic hilus using a 27-gauge needle. After waiting for one minute, the portal vein was ligated, the spleen was removed and the abdominal cavity was closed.

In vivo Effect of c-SF-25 MAb

The *in vivo* anti-tumor effect of c-SF-25 MAb was studied using the above described hepatic metastic model. Five days after the injection of LS 180 cells and anti-asialo GM1 antibody, mice were intravenously (iv.) injected with a single dose of c-SF-25 MAb (100 μg/mouse). As a control, an equal molar amount (67 μg/mouse) of $F(ab')_2$ fragment of c-SF-25 MAb was injected into other mice. Thirty days later (five weeks after the tumor cell injection), the mice were sacrificed and examined for metastatic spread in the liver and the presence of local abdominal tumors. Results were statistically significant when p was <0.05 by the chi-square test with the Yates correction.

In vivo Depletion of Murine Macrophage by Carrageenan

Since iota-carrageenan (iota-CGN; Sigma Chemical Co., St. Louis, MO) is toxic to macrophages (Ishizaka *et al., J. Immunol. 125*:2232-2235 (1980), this reagent was chosen as a macrophage depleting agent. However, native iota-CGN is not water-soluble at low temperatures and the sulfate groups contained in the iota-CGN are mitogenic (Id.) In order to prepare a

water soluble reagent and also to avoid non-specific stimulation of the immune system *in vivo*, iota-CGN was desulfated (DS-CGN) (Ishizaka *et al., J. Immunol. Method 124*:17-24 (1989)) and injected into the nude mice. For *in vivo* depletion of macrophages, 3 mg of DS-CGN dissolved in PBS was injected ip. every other day for 5 days (total of 9 mg/mouse). Preliminary experiments using flow cytometry, demonstrated that peritoneal murine macrophages were almost completely depleted by these injections and that peritoneal exudate cells positive for F4/80 decreased from 89.3% to 9.0%. Macrophages detected in murine peripheral blood were also depleted by the ip injection of DS-CGN and F4/80-positive cells decreased therein from 18.0% to 1.9%. These results show that DS-CGN was effective for the *in vivo* depletion of macrophages.

Survival Rate of Mice with Human Colon Cancer Adenocarcinoma Metastases

Mice with human colon adenocarcinoma metastases developed large hepatic tumors and all animals died within 6 to 7 weeks after the injection of tumor cells due to hepatic dysfunction. Mice were injected iv with c-SF-25 MAb (100 μg/mouse once a week for 4 weeks) starting 5 days after the intraportal injection of tumor cells. The survival rate was compared to the untreated control group using the algorithm of Lee and Desu (*Comput. Prog. Biomed. 2*:315-321 (1972)).

Properties of c-SF-25 MAb

The c-SF-25 showed identical physical properties to the murine MAb with respect to its immunoreactivity and association constant. The binding of ^{125}I-labeled c-SF-25 to the LS 180 cell line was completely inhibited by both cold murine SF-25 and cold c-SF-25 but not by B_2TT, a non-specific MAb (Figure 3). Thus, the chimeric and murine SF-25 MAb recognize the same epitope. Furthermore, the association constants of the chimeric and murine SF-25 MAbs as calculated by Scatchard analysis were 2.21 x 10^8/M and 1.36

x 10^8/M, respectively. These binding constants indicate that the antigen-antibody interaction is of high avidity and that the human/murine chimeric construct of the SF-25 MAb was identical to the murine antibody. Finally, the numbers of antibody binding sites per LS 180 cell were calculated to be approximately 2.5 x 10^5 for both the chimeric and original murine MAb.

ADCC Exhibited by Human Macrophages and NK-cells

ADCC produced by c-SF-25 MAb was studied using human PBL as effector cells (Figure 4A). ADCC was induced at various effector to target cell (E:T) ratios by c-SF-25 but not by murine SF-25 MAb. The specific lysis of colon adenocarcinoma cells by isolated subpopulations of cells was then examined. The purity of macrophages and NK-cells isolated from human PBL were >95% and >90%, respectively, when examined by flow cytometry. Both cell populations induced substantial ADCC against LS 180 cells in the presence of c-SF-25 MAb (Figure 4B) that was greater than that induced by the mixed cellular population of PBLs (Figure 4A).

Animal Model of Hepatic Metastic Disease

Hepatic metastases of human colon adenocarcinoma cells were established by injecting LS 180 cells into the portal vein of nude mice. The administration of anti-asialo GM1 is essential to establish hepatic metastases in nude mice (Takahashi *et al., Gastroenterology 96*:1317-1329 (1989)). However, when anti-asialo GM1 was injected iv. one or two days before tumor cell injection, hepatic tumors developed in only 60% and 40% of the mice, respectively. Therefore, all mice were simultaneously injected iv. with LS 180 cells and anti-asialo GM1 into the portal vein. All these mice developed macroscopic tumors in their livers as well as local abdominal tumors at the site of tumor cell injection.

Effect of Chimeric SF-25 MAb on Tumor Growth *in vivo*

All control mice developed extensive hepatic metastases 5 weeks after tumor cell injection (Group A in Table I). Large "cannon ball-like" multiple tumors developed in the livers of untreated mice (Figure 5A) and 74% of these untreated animals (14 of 19) developed multiple, isolated tumors larger than 5 mm in diameter (Table I).

A single iv. injection of c -SF-25 MAb produced a striking anti-tumor effect. The incidence of mice with hepatic metastases was significantly reduced from 100% (19 of 19) to 22% (2 of 9) ($P < 0.01$: c-SF-25 treated vs controls; Table I) and most of these mice were free of detectable hepatic tumors (Figure 5B). Two mice treated with c-SF-25 MAb developed hepatic tumors, but their tumor burden was substantially less than the controls and all tumors were less than 3mm. c-SF-25 MAb also inhibited local abdominal tumor growth ($P < 0.005$ compared to controls).

In contrast to the striking anti-tumor effect exhibited by c-SF-25 MAb, the $F(ab')_2$ fragment had little effect on colon adenocarcinoma cell growth. The mice treated with $F(ab')_2$ fragment developed multiple hepatic tumors larger than 5 mm in diameter (Group C in Table I) and were similar to control

mice (Figure 5C). The $F(ab')_2$ fragment had little effect on the local abdominal tumors (Table I).

Table I. *In Vivo* Anti-Tumor Effect of c-SF-25 MAb on Human Colon Adenocarcinoma Growth In Nude Mice

Group	A		B		C	
Treatment	None (n=19)		c-SF-25 MAb (n = 9)		$F(ab')_2$ of c-SF-25 (n = 10)	
Hepatic metastasis	19/19	(100%)	2/9	(22%)*	8/10	(80%)
<3 mm†	0/19	(0%)	2/2	(100%)	0/8	(0%)
3-5 mm	5/19	(26%)	0/2	(0%)	0/8	(0%)
>5 mm	14/19	(74%)	0/2	(0%)	8/8	(100%)
Local abdominal tumor	19/19	(100%)	4/9	(44%)§	9/10	(90%)
<3 mm	0/19	(0%)	4/4	(100%)	1/9	(11%)
3-5 mm	0/19	(0%)	0/4	(0%)	0/9	(0%)
>5 mm	19/19	(100%)	0/4	(0%)	8/9	(89%)

* $p<0.001$ vs. no treatment.
† Tumor size as measured by largest diameter of an individual tumor.
§ $p<0.005$ vs. no treatment.

In vivo Effect of c-SF-25 MAb after Macrophage Depletion

In order to test if macrophages were involved in the *in vivo* anti-tumor effect produced by c-SF-25 MAb, DS-CG was injected ip. to deplete murine macrophages after the injection of the LS 180 tumor cells. The DS-CGN basically did not affect the development of hepatic metastases (Group A v. Group B in Table II). The anti-tumor effect of c-SF-25 MAb (100 μg/mouse) on hepatic metastases was reduced by DS-CGN and hepatic metastases were present in all mice treated with c-SF-25 MAb (Group C, Table II). However, hepatic metastases of mice treated with c-SF-25 MAb and DS-CGN were still significantly smaller than those of untreated animals ($P<0.01$; Group C v. Group A in Table II). In contrast, the anti-tumor effect of c-SF-25 MAb on local abdominal tumors was completely blocked by DS-CGN and mice developed extrahepatic tumors similar in size to untreated mice (Group C v. Group A in Table II).

Analysis of Liver Infiltrating Lymphocytes

Three weeks after their LS 180 tumor cell injections, mice were injected iv. with c-SF-25 MAb (100 μg/mouse). The mice were sacrificed the following day. Flow cytometric analysis of liver infiltrating cells detected by anti-asialo-GM1 rabbit serum demonstrated a larger population of NK-cells in the liver (31%) than in peripheral blood (7%) in mice with liver tumors and also in normal animals (36% vs. 5%, respectively; Table III)). There was no significant difference in the macrophage population size, as determined by non-specific esterase activity between liver (25%) and peripheral blood (15%; Table III).

Table II. **Anti-tumor effect of c-SF-25 MAb after *in vivo* depletion of murine macrophages by desulfated iota-carrageenan**

Group	A		B		C	
DS-CGN*	-		+		+	
c-SF-25 MAb	-		-		+	
	(n = 13)		(n = 10)		(n = 10)	
Hepatic metastases	13/13	(100%)	10/10	(100%)	10/10	(100%)
<3 mm	0/13	(0%)	2/10	(20%)	5/10	(50%)
3-5 mm	5/13	(38%)	0/10	(0%)	4/10	(40%)
>5 mm	8/13	(62%)	8/10	(80%)	1/10	(10%)†
Local abdominal tumor	13/13	(100%)	8/10	(80%)	9/10	(90%)
<3 mm	0/13	(0%)	0/8	(0%)	0/9	(0%)
3-5 mm	0/13	(0%)	0/8	(0%)	0/9	(0)%)
>5 mm	13/13	(100%)	8/8	(100%)	9/9	(100%)

* Desulfated iota-carrageenan (DS-CGN).

† $p < 0.01$ vs. no treatment.

Table III. Analysis of Liver Infiltrating Cells

Hepatic metastasis		+	-
Liver infiltrating cells	Anti-asialo GM1 positive	31.0%	36.0%
	Non-specific esterase positive	25.0%	20.0%
Peripheral blood cells	Anti-asialo GM1 positive	7.0%	5.0%
	Non-specific esterase positive	15.0%	18.0%

ADCC Produced by Murine Macrophages and NK-Cells

ADCC was induced by c-SF-25 MAb using thioglycolate-elicited murine macrophages as the effector cells at various E:T ratios (Figure 6A). Cytotoxicity was also produced when [^{3}H]methyl-thymidine labeled LS 180 cells were used as target cells in a longer term cytotoxicity assay (12-24h; data not shown). ADCC was induced by murine splenocytes in the presence of c-SF-25 MAb (Figure 6B).

NK-cells were isolated from these murine splenocytes by discontinuous density gradient centrifugation. The purity of these NK-cells, as determined by flow cytometry, was over 90%. These purified murine NK-cells demonstrated ADCC in the presence of c-SF-25 MAb. (Figure 6C).

Effect of c-SF-25 MAb on Animal Survival

All control mice died within 6 weeks after tumor cell injection due to massive hepatic metastases and liver dysfunction (Figure 7). In contrast, all mice administered c-SF-25 MAb survived more than 6 weeks and 60% of them survived more than 9 weeks. Median survival of the control and treated groups were 37 $\pm$ 4 days and 79 $\pm$ 35 days, respectively. The improved survival rate of the c-SF-25 MAb treated animals was highly significant ($P<0.0002$).

Properties of Chimeric Antibodies

Binding studies were performed. The c-SF-25 MAb association constant was 2.2×10^8 M^{-1} . The number of SF-25 and binding sites per cell were 2.3×10^5/cell when studied at 4°C using LS 180 cells as the antigen source.

Functional studies with chimeric SF-25 MAbs were performed in order to determine if the human IgG1 isotype could induce ADCC activity by murine effector cells. ADCC was induced at various effector to target cell (E:T) ratios greater than 12.5:1 only in the presence of chimeric MAbs (20μg/ml; Figure 8). Murine splenocytes alone had no cytotoxicity against LS 180 cells.

In vivo Effect of Chimeric Antibodies

An animal model of hepatic metastases was employed to test the anti-tumor effects of chimeric SF-25 MAb. All control mice injected with LS-180 cells as previously described developed both hepatic and local abdominal tumors (Table 4). The typical tumor growth pattern in a control animal sacrificed 5 weeks after tumor cell injection showed large "cannon-ball" like tumors throughout the liver.

A single intravenous injection of chimeric SF-25 MAb substantially inhibited LS-180 tumor growth. The percent of mice bearing hepatic metastases was reduced from 100% to 22% ($P < 0.001$: vs. controls; Table 4) and most were entirely free of detectable hepatic disease. Two animals in the SF-25 MAb treatment group developed hepatic tumors at 5 weeks, but the size of these tumors was considerably smaller than in the controls (Table 4). Local abdominal tumors were also reduced in the SF-25 treatment group (100% to 44%, $P < 0.025$; Table 4).

Table 4. ***In vivo*** **anti-tumor effect of chimeric SF-25 MAb on human colon adenocarcinoma growth in nude mice.**

Treatment	None (n = 13)		c-SF-25 MAb (n = 9)	
Hepatic metasteses	13/13	(100%)	2/9	(22%)*
< 3 mm†	0/13	(0%)	2/9	(22%)
3-5 mm	5/13	(38%)	0/9	(0%)
> 5 mm	8/13	(62%)	0/9	(0%)
Local abdominal tumor	13/13	(100%)	4/9	(44%)§

* $P < 0.01$ vs. no treatment.
† Largest tumor size as measured by diameter of individual tumors.
§ $P < 0.025$ vs. no treatment.

Chimeric Antibody Binding Studies

Since the *in vitro* and *in vivo* experiments were performed at 4°C and under physiological conditions (37°C), respectively, direct binding assays of ^{125}I-labeled SF-25 MAbs to LS 180 tumor cells were performed at different temperatures. The specific binding of SF-25 to the tumor cell surface was significantly greater at 37°C than at 4°C ($P < 0.001$; Figure 9).

The binding of SF-25 MAb to the tumor cell surface was relatively stable both at 4°C and at 37°C (Table 5).

Table 5. Flow cytometric analysis of monoclonal antibody binding to tumor cells

		Mean percent of positive staining cells (%)			
		0 min	15 min	30 min	120 min
Murine SF-25	4°C	91.4	90.2	90.7	91.8
	37°C	91.4	90.0	82.4	66.4

Direct binding investigations using different MAbs demonstrated that at 37°C, the specific binding of both GA733 and 323/A3 MAbs increased (Figure 10). Because the binding of GA733 and 323/A3 appeared to be stable at 37°C, these data indicate that the antigen recognized by both MAbs (GA733 and 323/A3) was not shed from the cell surface.

To confirm and extend these observations, LS 180 tumor tissue was placed in various fixatives to immobilize the antigen in the membrane. ^{125}I-labeled GA733 and 323/A3 MAbs reacted with LS 180 tumor tissue at 4°C. Furthermore, there was no temperature effect on the binding of GA733 and 323/A3 MAbs to LS 180 derived tumor tissue. Identical results were obtained when LS 180 tumors were fixed with 2% paraformaldehyde or 4% formaldehyde at various times and temperatures.

Association Constants of Monoclonal Antibodies

The binding association constants of chimeric and murine SF-25, GA733 and 323/A3 MAb remained unchanged at 37°C compared to 4°C (Table 6).

ADCC Induced by Various Concentrations of Chimeric Antibodies

The ADCC activities produced by chimeric SF-25 at various antibody concentrations were studied. Chimeric SF-25 MAbs induced ADCC mediated by murine NK-cells purified from a splenocyte derived population at 37°C in the presence of low and high concentrations of antibody (20μg/ml; Figure 11A). Thioglycollate elicited macrophages mediated ADCC at both low and high concentrations of chimeric SF-25 MAbs (Figure 11B).

Table 6. Association constants of monoclonal antibodies

MAb	4°C K_A ($X10^8M^{-1}$)	37°C K_A ($X10^8M^{-1}$)
chimeric SF-25	2.21	4.86
murine SF-25	1.42	1.26
murine GA73-3	0.42	0.65
murine 323A3	0.39	0.68

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 1, line 10.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
08 December 1991	HB 9599

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

Murine Hybridome Cell Line, SF-25

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Form PCT/RO/134 (July 1992)

WHAT IS CLAIMED IS:

1. A polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human immunoglobulin chain, both said sequences in operable linkage with each other.

2. The molecule of claim 1 wherein said chain is a heavy chain.

3. The molecule of claim 1 wherein said chain is a light chain.

4. The molecule of claim 1 which is a recombinant DNA molecule.

5. The molecule of claim 4 which is in double stranded DNA form.

6. The molecule of claim 5 which is an expression vehicle.

7. The molecule of claim 6 wherein said vehicle is a plasmid.

8. A prokaryotic host transformed with the molecule of claim 1.

9. The host of claim 8 which is a bacterium.

10. A eukaryotic host transfected with the molecule of claim 1.

11. The host of claim 10 which is a yeast cell or a mammalian cell.

12. A chimeric immunoglobulin heavy chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

13. A chimeric immunoglobulin light chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

14. A chimeric antibody molecule comprising two light chains and two heavy chains, each of said chains comprise at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody given the ATCC designation HB 9599, or a fragment or derivative of said chimeric antibody.

15. The chimeric immunoglobulin heavy chain of claim 12 wherein said heavy chain is detectably labeled.

16. The chimeric immunoglobulin light chain of claim 13 wherein said light chain is detectably labeled.

17. The antibody, fragment or derivative thereof, of claim 14 wherein said antibody, fragment or derivative thereof, is detectably labelled.

18. The molecule of any of claims 15, 16 or 17 wherein said detectable label is an enzyme label.

19. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a radioisotopic label.

20. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a nuclear magnetic resonance contrasting agent.

21. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a nonradioactive isotopic label.

22. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a fluorescent label.

23. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a toxin label.

24. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a chemiluminescent label.

25. The chimeric immunoglobulin heavy chain of claim 12 wherein said heavy chain is conjugated with a therapeutic molecule.

26. The chimeric immunoglobulin light chain of claim 13 wherein said light chain is conjugated with a therapeutic molecule.

27. The antibody, fragment or derivative thereof, of claim 14 wherein said antibody, fragment or derivative thereof, is conjugated with a therapeutic molecule.

28. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is a toxin molecule.

29. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is radionuclide.

30. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is cytotoxic drug.

31. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is photo-activatable toxin.

32. A process for preparing a chimeric immunoglobulin heavy chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599,comprising:

(a) culturing a host capable of expressing said heavy chain under culturing conditions,

(b) expressing said heavy chain; and

(c) recovering said heavy chain from said culture.

33. A process for preparing a chimeric immunoglobulin light chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising:

(a) culturing a host capable of expressing said light chain under culturing conditions;

(b) expressing said light chain; and

(c) recovering said light chain from said culture .

34. A process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the hybridoma cell line given the ATCC designation HB 9599 comprising:

a. culturing a host capable of expressing said light chain under culturing conditions, expressing said light chain, and recovering said light chain from said culture;

(b) separately culturing a host capable of expressing said heavy chain under culturing conditions, expressing said heavy chain, and recovering said heavy chain from said culture; and

(c) associating said recovered heavy chain and light chain, thereby preparing said chimeric immunoglobulin, fragment or derivative.

35. A process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising:

(a) co-culturing a host capable of expressing said heavy chain with a host capable of expressing said light chain under culturing conditions;

(b) expressing said heavy chain and said light chain;

(c) permitting said heavy chain and said light chain to associate into said chimeric immunoglobulin, fragment or derivative; and

(d) recovering said chimeric immunoglobulin, fragment or derivative from said culture.

36. A process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the hybridoma cell line given the ATCC designatio HB 9599, comprising:

(a) culturing a host capable of expressing said heavy chain and said light chain under culturing conditions;

(b) expressing said chimeric immunoglobulin, fragment or derivative and

(c) recovering from said culture said chimeric immunoglobulin, fragment or derivative.

37. The process of any of claims 32-36 wherein said host is prokaryotic.

38. The process of any of claims 32-36 wherein said host is eukaryotic.

39. An immunoassay method for detecting an antigen capable of binding to the chimeric SF-25 monoclonal antibody in a sample comprising:

(a) contacting said sample with the detectably labeled antibody, fragment or derivative of any of claims 17-23 or 24; and

(b) detecting said antigen by detecting the binding of said antigen to said antibody, fragment or derivative thereof.

40. An imaging method for detecting a tissue antigen capable of binding to the chimeric SF-25 monoclonal antibody, comprising:

(a) contacting the detectably labeled antibody, fragment or derivative of any of claims 17-23 or 24 with said tissue; and

(b) detecting said antigen.

41. The method of claim 40, wherein said contacting occurs *in vivo* within the body of an animal.

42. The method of claim 41, wherein said animal is a human being.

43. The method of claim 40, wherein said contacting occurs *in vitro* outside the body of an animal.

44. The method of claim 40 wherein said tissue is colon adenocarcinoma tissue.

45. The method of claim 40 wherein said tissue is hepatic metastatic tissue derived from colon adenocarcinoma tissue.

46. The use of an effective dose of a chimeric SF-25 monoconal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for the killing of cells expressing an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody.

47. The use of claim 46, wherein said killing occurs *in vivo* within the body of an animal.

48. The use of claim 47, wherein said animal is a human being.

49. The use of claim 46, wherein said cells are of endodermal origin.

50. The use of claim 49, wherein said cells are colon adenocarcinoma cells.

51. The use of claim 50, wherein said cells are cells of hepatic metastases which are derived from said colon adenocarcinoma cells.

52. The use of claim 46, wherein said killing occurs by antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, radiation cytotoxicity, cytotoxic drug action, or toxin action.

53. The use of claim 46,wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

54. The use of claim 46, wherein said fragment is a single chain antibody binding protein.

55. The use of claim 46, wherein said chimeric SF-25 monoclonal antibody is a humanized SF-25 monoclonal antibody.

56. The use of claim 55, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of variable domains of an antibody which recognizes the SF-25 antigen defined by the murine SF-25 antibody.

57. The use of an effective dose of a chimeric SF-25 monoclonal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for treating an animal having a tumor expressing an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody.

58. The use of claim 57, wherein said animal is a human being.

59. The use of claim 57, wherein said tumor is of endodermal origin.

60. The use of claim 59, wherein said tumor is a colon adenocarcinoma.

61. The use of claim 60, wherein said tumor is a hepatic metastatic tumor which is derived from said colon adenocarcinoma.

62. The use of claim 57, wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

63. The use of claim 57, wherein said fragment is a single chain antibody binding protein.

64. The use of claim 57, wherein said chimeric SF-25 monoclonal antibody is a humanized SF-25 monoclonal antibody.

65. The use of claim 64, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of variable domains of an antibody which recognizes the SF-25 antigen defined by the murine SF-25 antibody.

66. The use of an effective dose of a chimeric SF-25 monoclonal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for treating an animal which has pancreatic carcinoma or other pancreatic cancer, which expresses an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody.

67. The use of claim 66, wherein said antibody is a chimeric SF-25 monoclonal antibody.

68. The use of claim 66, wherein said antibody is a humanized SF-25 monoclonal antibody.

69. The use of claim 68, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of the variable domains of an antibody which recognizes the SF-25 antigen as defined by murine SF-25 antibody.

70. The use of claim 66, wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

71. The use of claim 66, wherein said fragment is a single chain antibody binding protein.

72. The use of claim 66, wherein said treatment occurs *in vivo* within the body of the animal.

73. The use of claim 72, wherein said animal is a human.

74. The use of an effective dose of a chimeric SF-25 monoclonal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for treating an animal having cancer which expresses an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody, with the cancer selected from the group consisting of colon adenocarcinoma, hepatocellular carcinoma, cholangiocellular carcinoma, gastric adenocarcinoma, rectal adenocarcinoma, breast adenocarcinoma, bladder adenocarcinoma, squamous cell carcinoma of the lungs, adenocarcinoma of the lungs, large cell carcinoma of the lungs, small cell carcinoma of the lungs, lymphoproliferative disease, myeloproliferative disease, lymphoma, leukemia, kidney carcinoma, ovary adenocarcinoma, cervical carcinoma, uterine endometrial adenocarcinoma, liver hepatoma, choriocarcinoma, malignant meloma, including the primary tumors or metastases or micrometastases of these diseases.

75. The use of claim 74, wherein said antibody is a chimeric SF-25 monoclonal antibody.

76. The use of claim 74, wherein said antibody is a humanized SF-25 monoclonal antibody.

77. The use of claim 76, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of the variable domains of an antibody which recognizes the SF-25 antigen as defined by murine SF-25 antibody.

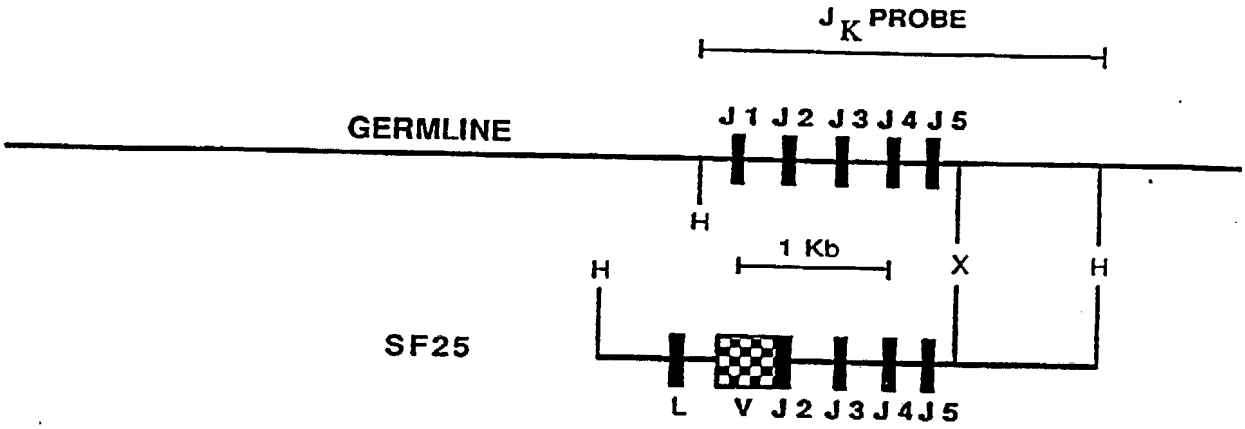
78. The use of claim 74, wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

79. The use of claim 74, wherein said fragment is a single chain antibody protein.

80. The use of claim 74, wherein said treatment occurs *in vivo* within the body of the animal.

81. The use of claim 74, wherein said animal is a human being.

A.


J K PROBE
GERMLINE
J1 J2 J3 J4 J5
H
1 Kb
H
X
H
SF25
L V J2 J3 J4 J5

B.

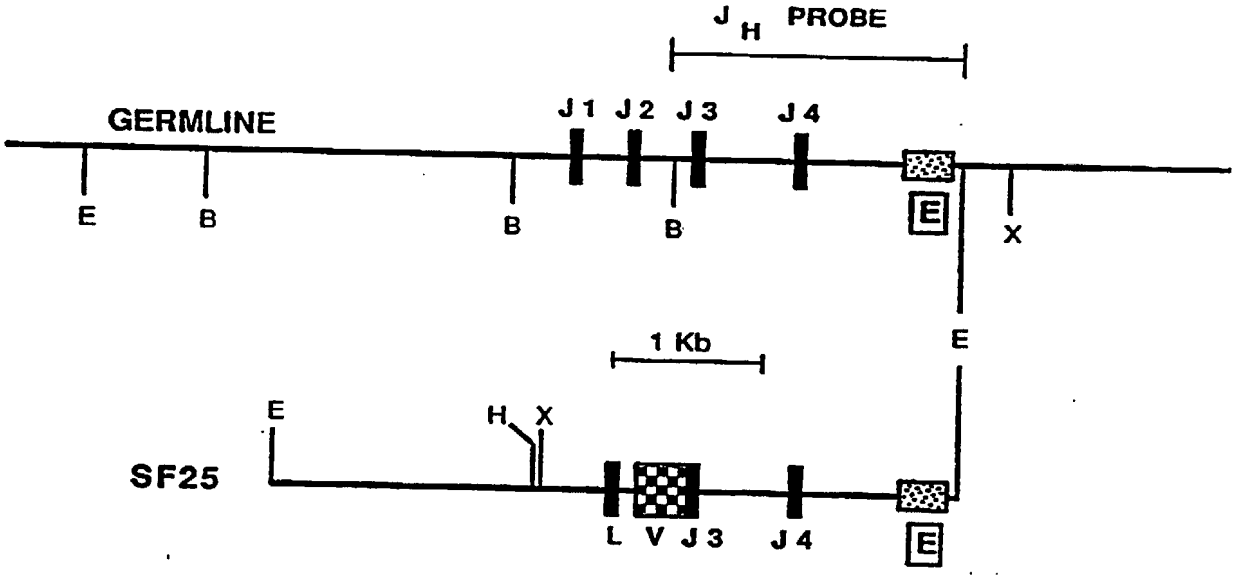
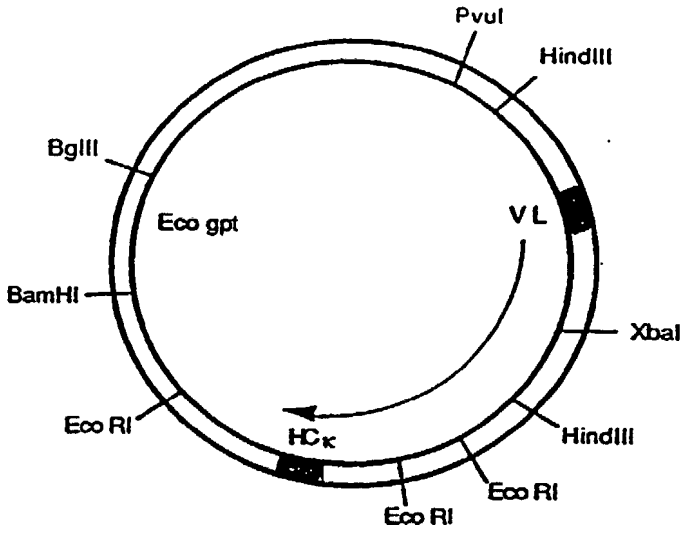
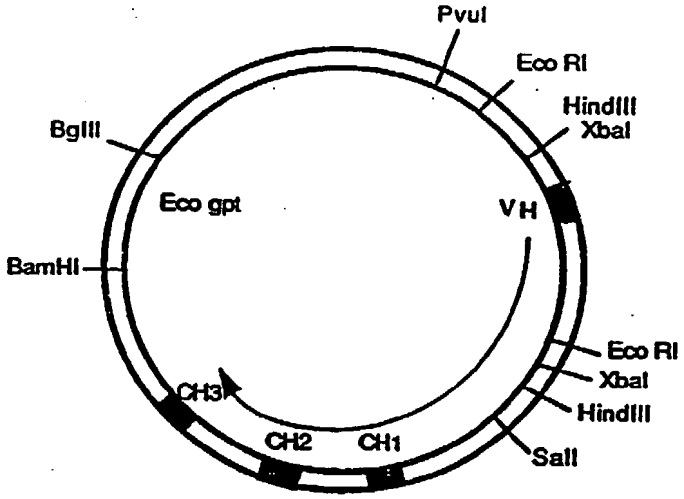

J H PROBE
GERMLINE
J1 J2 J3 J4
E
B
B
B
E
X
E
1 Kb
E
H X
SF25
L V J3 J4
E

Figure 1



pSF25Kapgpt

A.



pSF25G1apgpt

B.

Figure 2

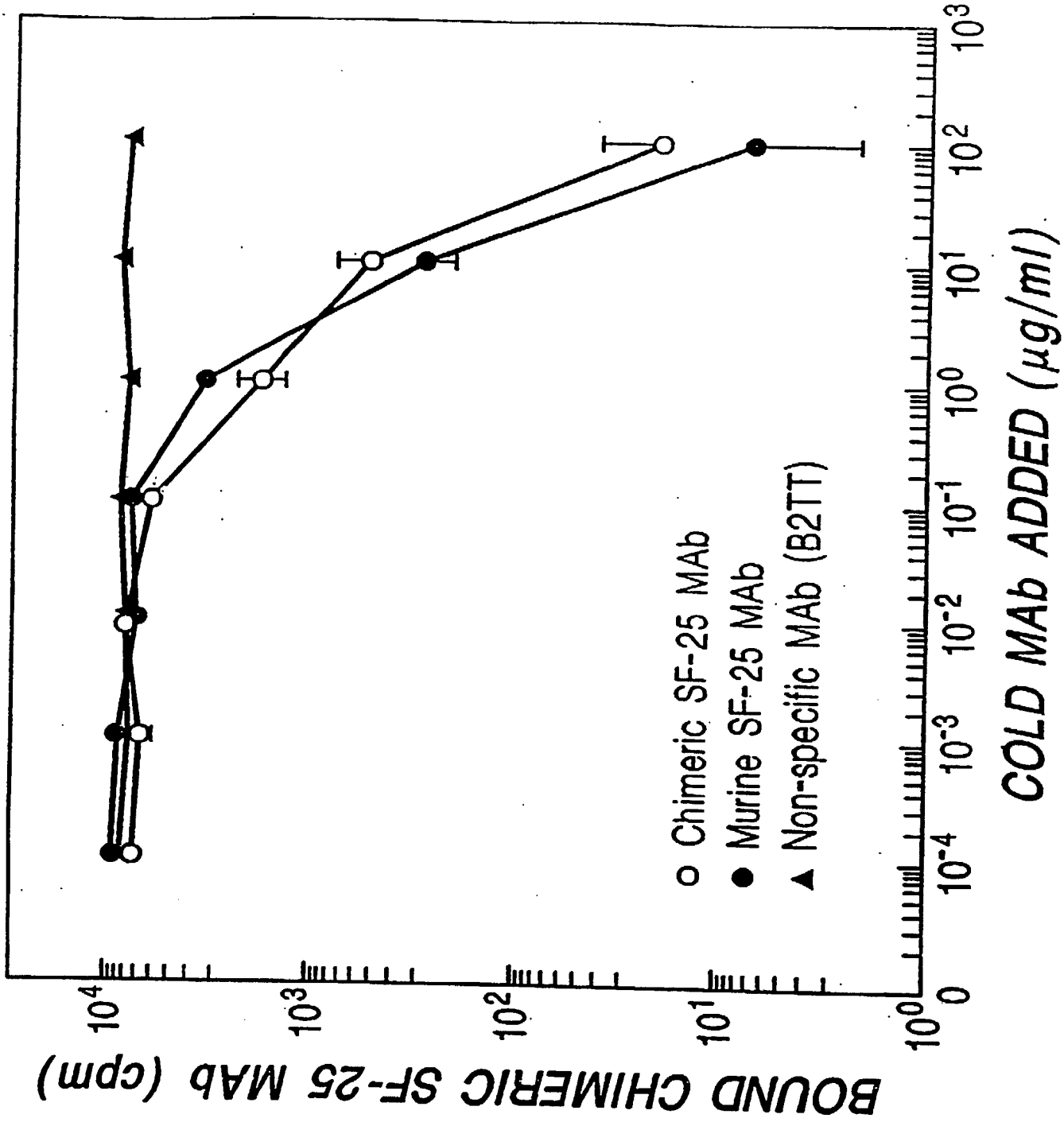
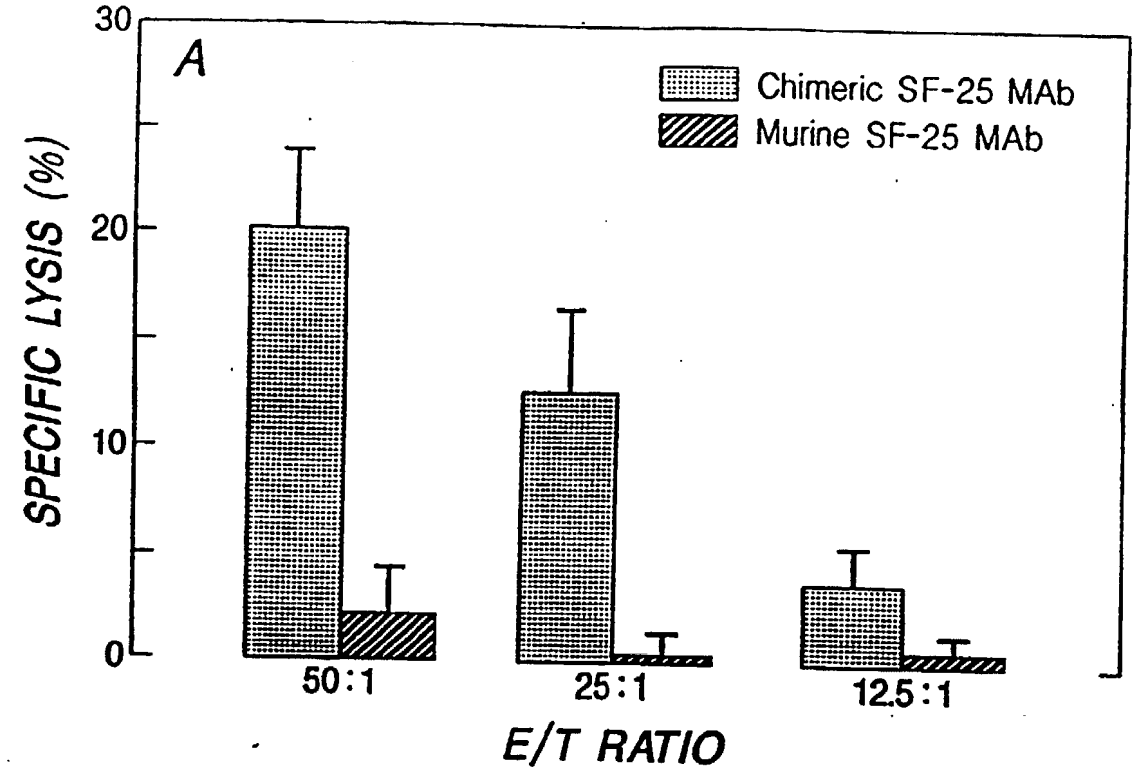

BOUND CHIMERIC SF-25 MAb (cpm)
COLD MAb ADDED (μg/ml)
o Chimeric SF-25 MAb
● Murine SF-25 MAb
▲ Non-specific MAb (B2TT)
10^0
10^1
10^2
10^3
10^4
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
10^2
10^3

Figure 3


A
Chimeric SF-25 MAb
Murine SF-25 MAb
SPECIFIC LYSIS (%)
30
20
10
0
50:1
25:1
12.5:1
E/T RATIO

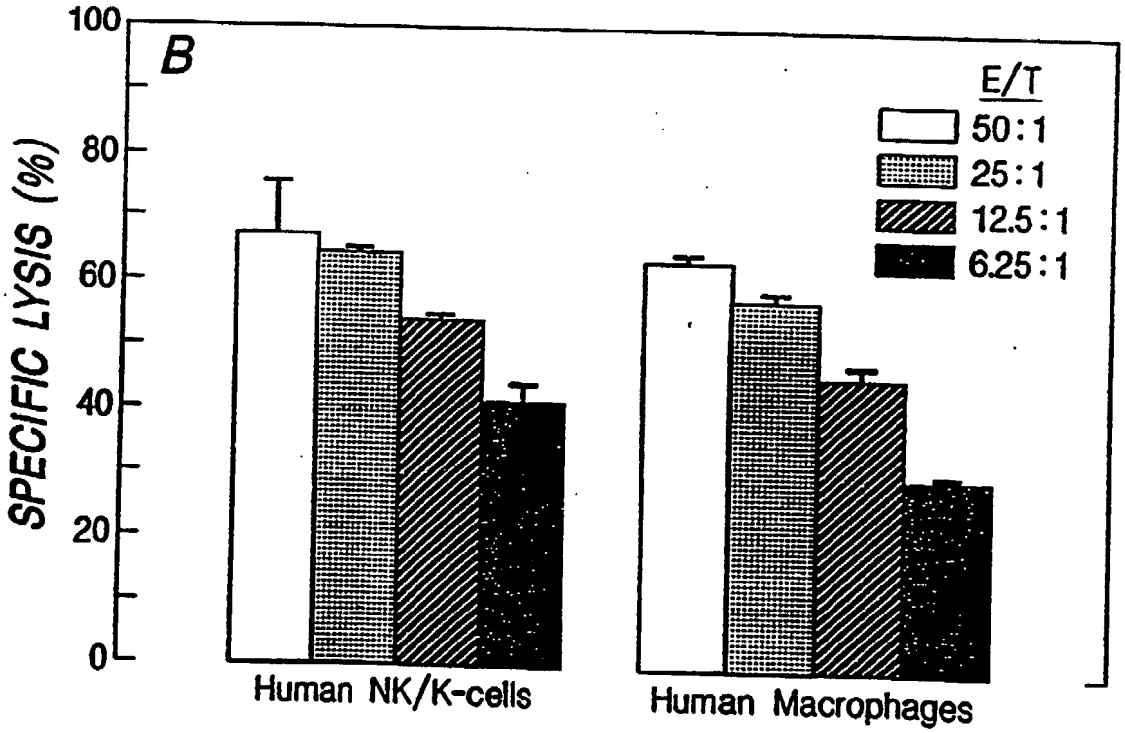

B
E/T
50:1
25:1
12.5:1
6.25:1
SPECIFIC LYSIS (%)
100
80
60
40
20
0
Human NK/K-cells
Human Macrophages

Figure 4

A. No Treatment

B. Chimeric SF-25 MAb

C. $F(ab')_2$ of Chimeric SF-25 MAb

Figure 5

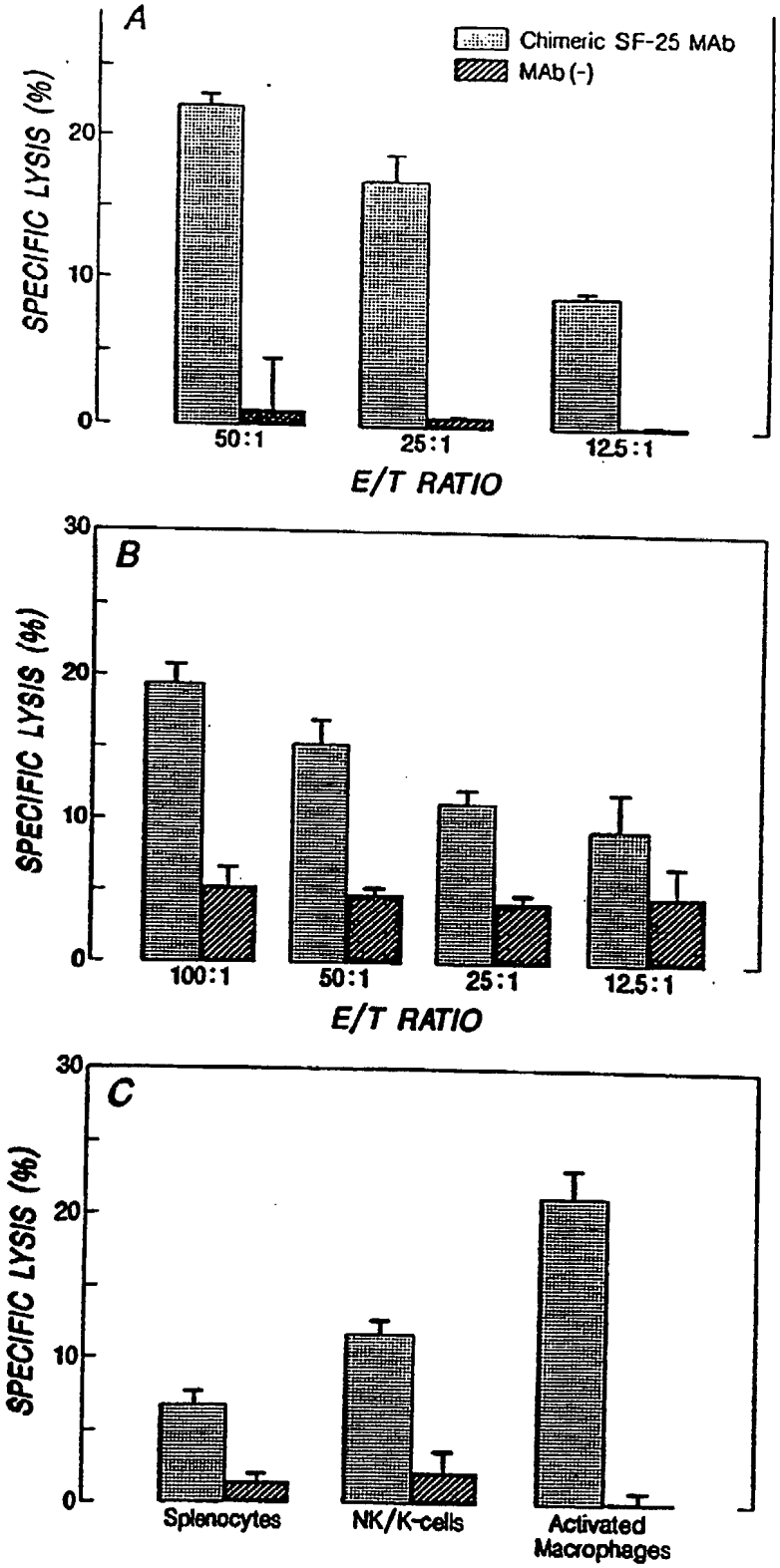

A
Chimeric SF-25 MAb
MAb (-)
SPECIFIC LYSIS (%)
0
10
20
50:1
25:1
12.5:1
E/T RATIO
B
SPECIFIC LYSIS (%)
0
10
20
30
100:1
50:1
25:1
12.5:1
E/T RATIO
C
SPECIFIC LYSIS (%)
0
10
20
30
Splenocytes
NK/K-cells
Activated Macrophages

Figure 6

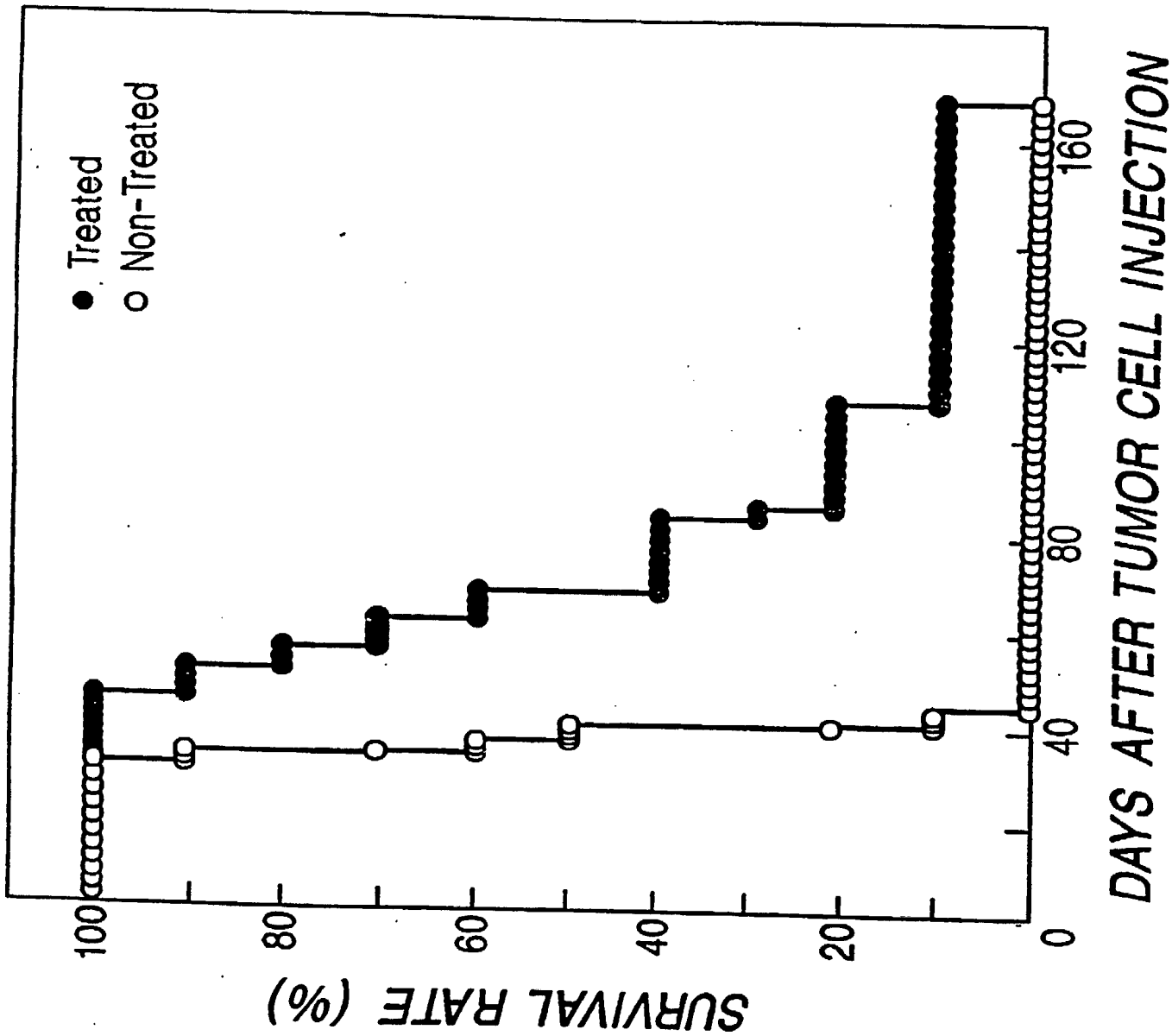

Treated
Non-Treated
SURVIVAL RATE (%)
100
80
60
40
20
0
40
80
120
160
DAYS AFTER TUMOR CELL INJECTION

Figure 7

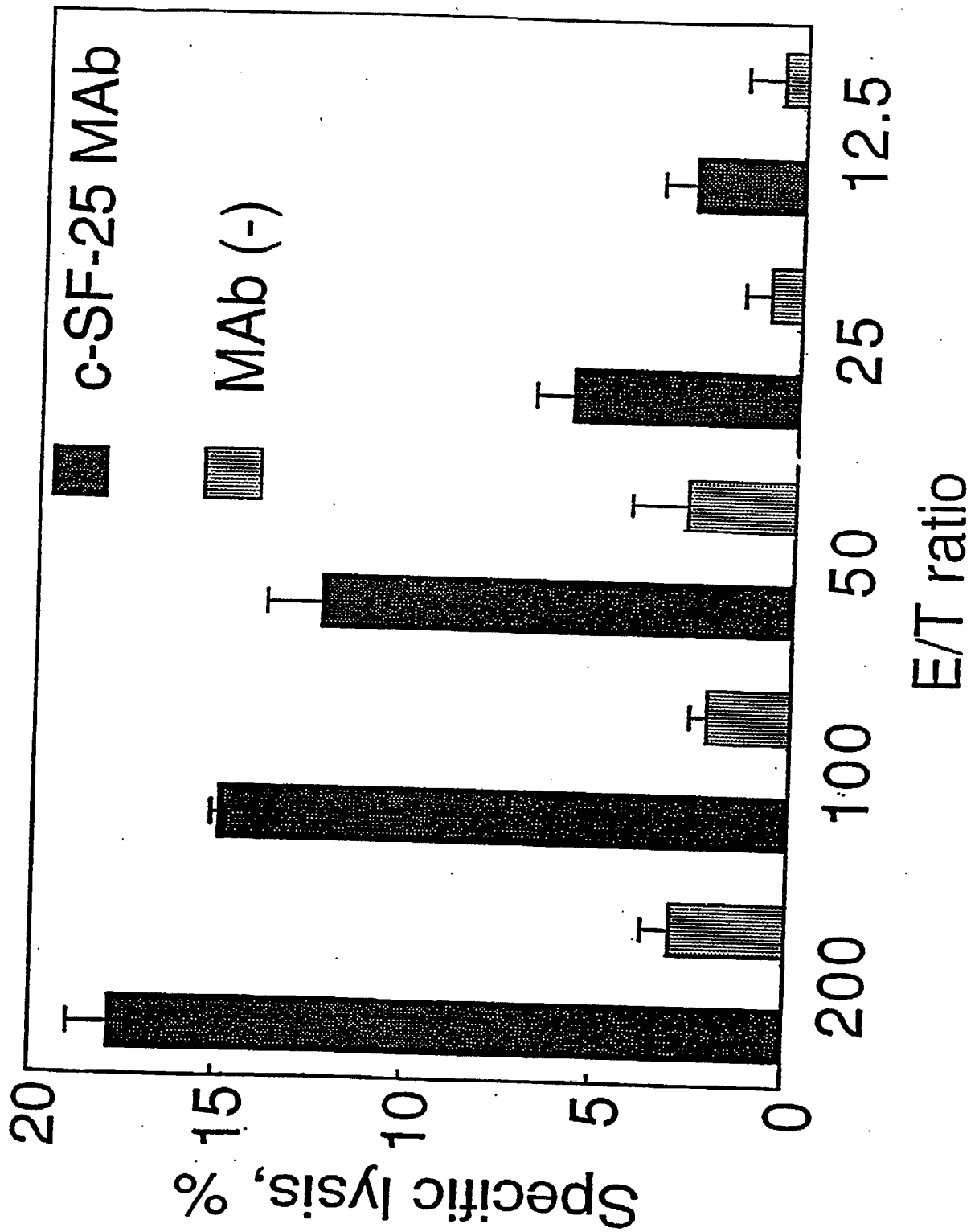

c-SF-25 MAb
MAb (-)
Specific lysis, %
0
5
10
15
20
E/T ratio
200
100
50
25
12.5

Figure 8

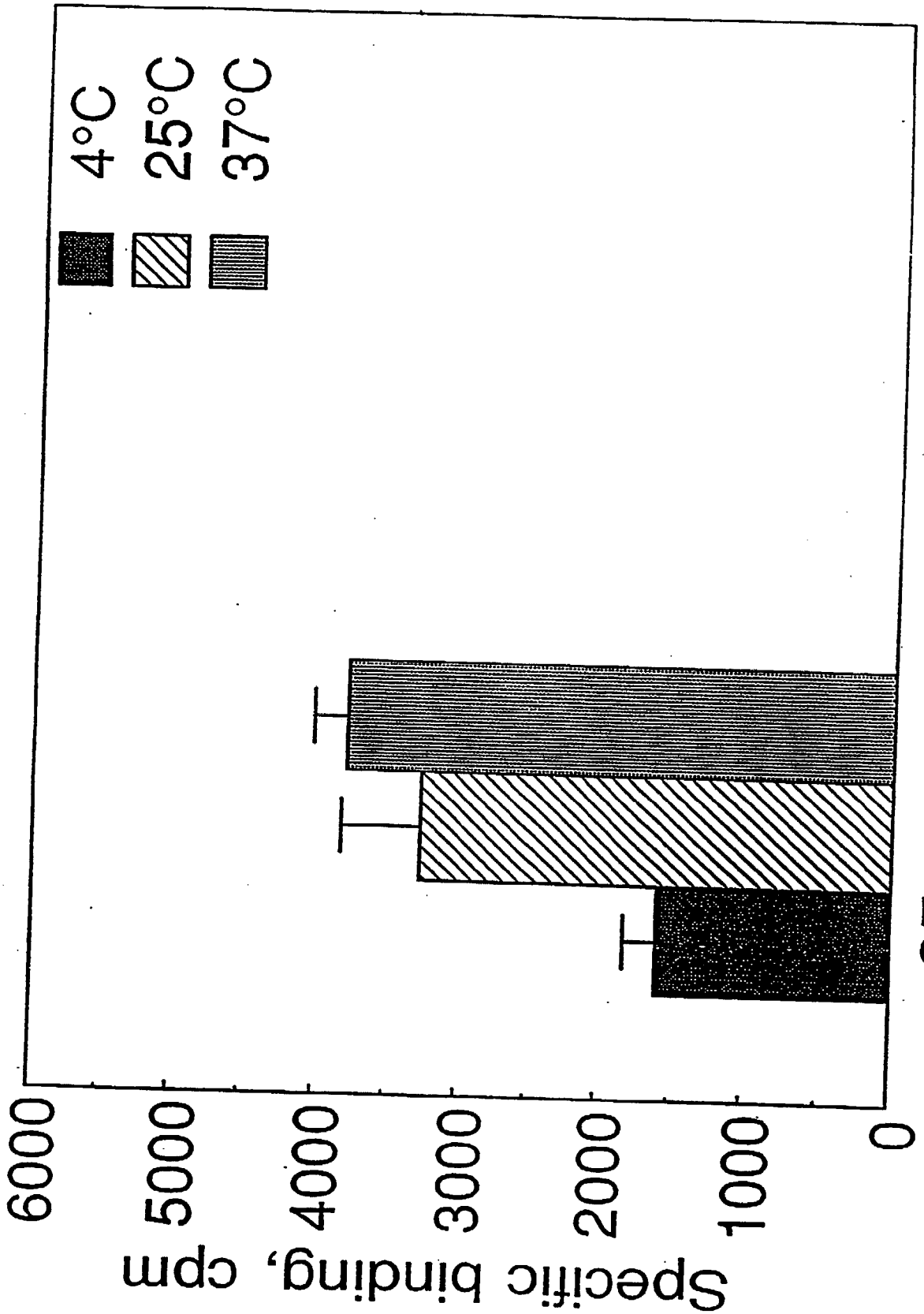

4°C
25°C
37°C
Specific binding, cpm
6000
5000
4000
3000
2000
1000
0
c-SF-25 MAb

Figure 9

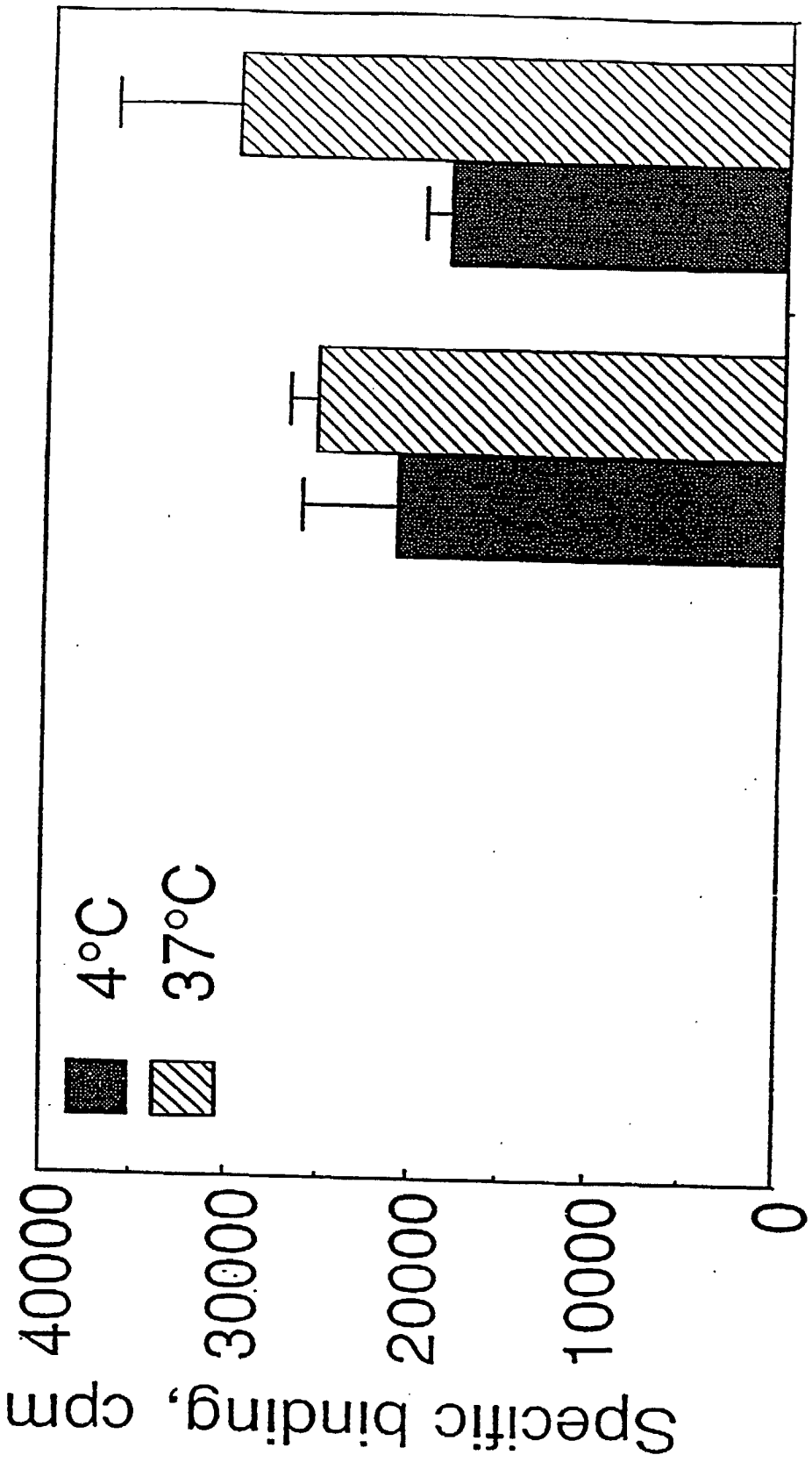
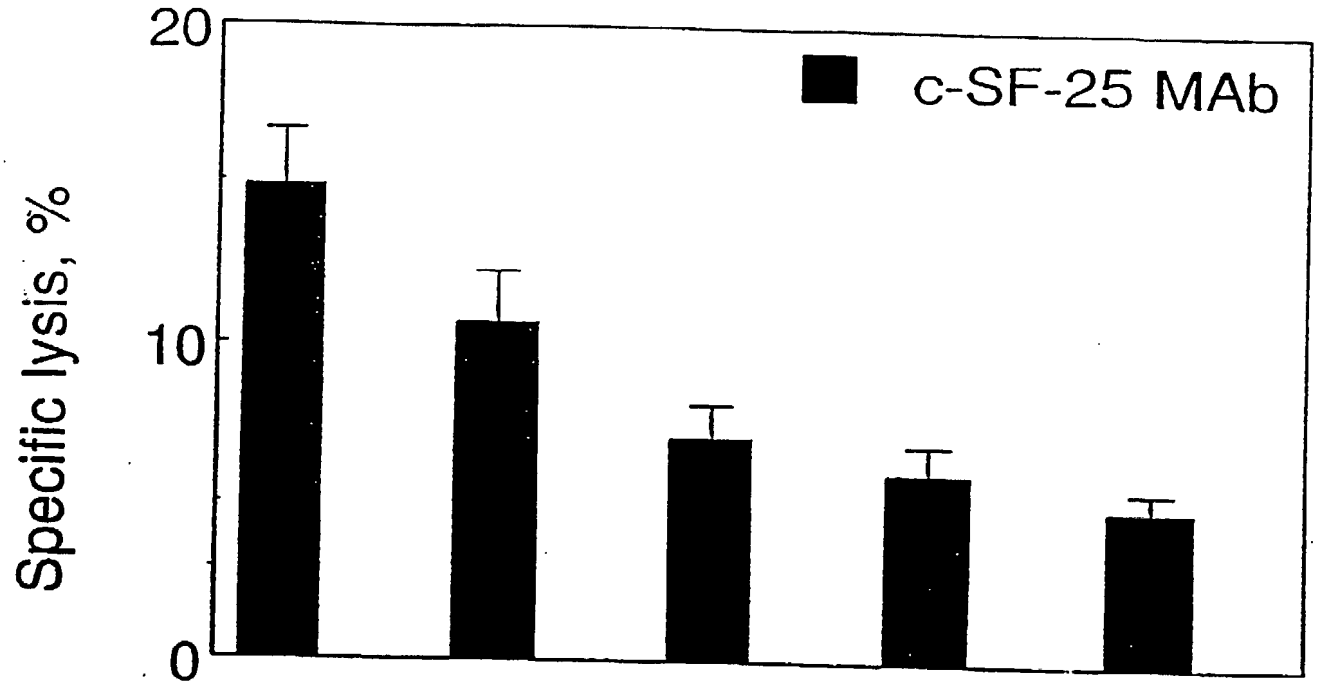

Specific binding, cpm
40000
30000
20000
10000
0
4°C
37°C
GA733
323/A3

Figure 10


20
10
0
Specific lysis, %
c-SF-25 MAb

B.

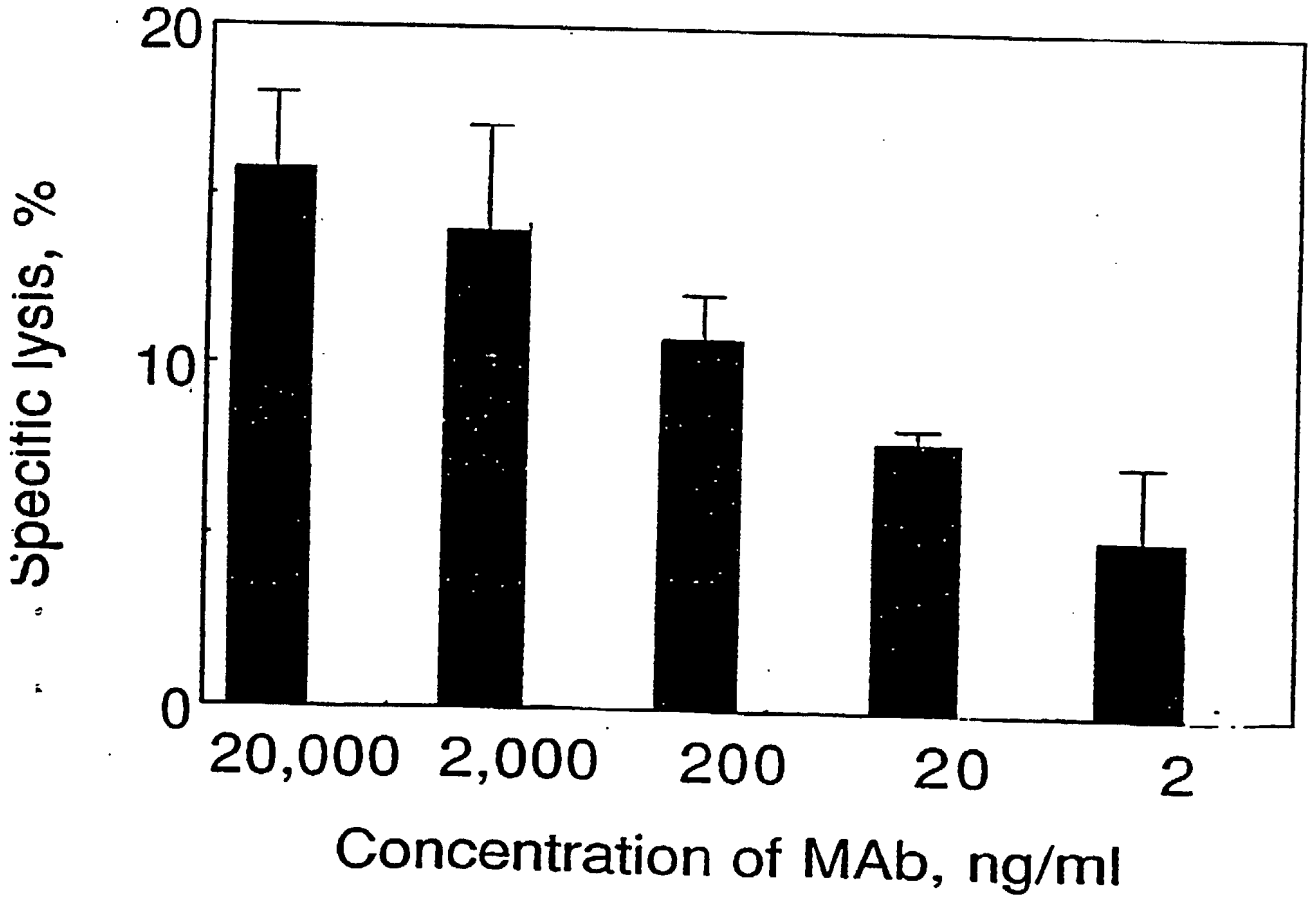

20
10
0
Specific lysis, %
20,000
2,000
200
20
2
Concentration of MAb, ng/ml

Figure 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08109

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/27; 435/320.1, 240.2, 252.2, 7.23, 70.21, 172.2; 530/387.3, 388.25, 391.1, 391.7; 424/1.1, 85.8, 85.91

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Cas Online, APS, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Gastroenterology, Volume 96, No.5, Part 1, issued 1989, H. Takahashi et al., "Radioimmunolocalization of Hepatic and Pulmonary Metastasis of Human Colon Adenocarcinoma", pages 1317-29, especially page 1317.	1-65, 74-81
Y	Annals New York Academy of Sciences, Volume 507, issued 1988, S. L. Morrison et al., "Genetically Engineered Antibody Molecules and Their Application" pages 187-198, see entire document.	1-65, 74-81
Y	Methods in Enzymology, Volume 178, issued 1989, M. Better et al., "Expression of Engineered Antibodies and Antibody Fragments in Microorganisms", pages 476-496, see entire document.	8-65, 74-81
Y	Proceedings of the National Academy of Sciences USA, Volume 86, issued December 1989, C. Queen et al., "A Humanized Antibody that Binds to the Interleukin 2 Receptor", pages 10029-10033, see especially pages 10029-10031.	12-65, 74-81

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08109

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biotechnology, Volume 9, issued February 1991, G. T. Davis et al., "Single Chain Antibody (SCA) Encoding Genes: One Step Construction and Expression in Eukaryotic Cells", pages 165-169, see entire document.	54, 63, 71, 79
Y	A. Pincera et al., "Monoclonal Antibodies '84: Biological and Clinical Applications", published 1985 by Editrice Kurtis s.r.l. see pages 475-506, especially pages 475-486.	23, 25-31, 40-65, 74-81
Y	Clinical Chemistry, Volume 27 No. 11, issued 1981, E. D. Sevier et al., "Monoclonal Antibodies in Clinical Immunology" pages 1797-1806, see especially pages 1800-1802.	15-19, 21, 22, 24, 39, 43
Y	US, A, 4,735,210 (Goldenberg) 05 April 1988, col. 14-15.	20, 39-45
Y	Vogel et al., "Immunoconjugates. Antibody Conjugates in Radioimaging and Therapy of Cancer", published 1987 by Oxford University Press, see pages 259-280.	17, 19, 25-27, 29, 39-65, 74-81

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08109

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07H 15/12; C12N 15/00, 5/10, 1/20, 15/02; C07K 15/28; C12P 21/08; A61K 43/00, 39/395; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/27; 435/320.1, 240.2, 252.2, 7.23, 70.21, 172.2; 530/387.3, 388.25, 391.1, 391.7; 424/1.1, 85.8, 85.91

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(54) Title: SF-25 ANTIBODIES, ESPECIALLY CHIMERIC ANTIBODIES, WITH SPECIFICITY FOR THE HUMAN TUMOR SF-25 ANTIGEN, METHODS FOR THEIR PRODUCTION, AND USES THEREOF

(57) Abstract

The present invention concerns SF-25 monoclonal antibodies, especially chimeric antibodies, and derivatives and fragments thereof, having specificity to the SF-25 antigen of human tumor cells, methods of their production, pharmaceutical compositions containing them, and uses therefor.

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TITLE

SF-25 ANTIBODIES, ESPECIALLY CHIMERIC ANTIBODIES, WITH SPECIFICITY FOR THE HUMAN TUMOR SF-25 ANTIGEN, METHODS FOR THEIR PRODUCTION, AND USES THEREOF

I. Cross Reference to Related Applications

This application is related to United States Patent Applications Serial Nos.: 07/765,350, 07/765,351 and 07/765,612, all three of which were filed on September 25, 1991; 07/203,198 filed on June 7, 1988; and 07/130,777 (now abandoned) which was filed on December 9, 1987.

II. Field of the Invention

The present invention relates to SF-25 antibodies, especially chimerized antibodies, and fragments and derivatives thereof, which have specificity for the human tumor SF-25 antigen and especially to humanized chimeric SF-25 antibodies; to methods of producing these SF-25 antibodies, fragments, and derivatives thereof, using recombinant DNA technology; to the nucleotide and protein sequences coding for these SF-25 antibodies, fragments, and derivatives; to methods of obtaining and manipulating these sequences; processes for the manufacture of pharmaceutical compositions containing these SF-25 antibodies, fragments and derivatives thereof; and also the uses thereof.

III. Background of the Invention

A. Monoclonal Antibodies

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $F(ab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the free end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assays, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realization of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies (MAb) of defined and exquisite specificity (Kohler *et al., Nature* 265:295-497 (1975)).

MAbs produced from hybridomas are already widely used in basic research, are being tested in the treatment of human diseases, including cancer, viral and microbial infections, other diseases and disorders of the immune system. However, most MAbs have been produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the rodent MAb and will either remove it entirely or at least reduce its

effectiveness. In practice, MAbs of rodent origin may not be used in a patient for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Since MAbs of rodent origin are distinguished from human proteins, they are cleared rapidly from the human circulation. In addition, most mouse MAbs are not cytocidal against neoplastic cells in humans because the murine MAbs do not participate in human complement or cell-mediated cytotoxicity (Waldmann, T.A., *Science 252*:1657-1662 (1991); Robinson *et al.*, *Hum. Antibod. Hybridomas 2*:84-93 (1991); Shin, S.U., *Biother. 3*:43-53 (1991); Ahmad *et al.*, *Mol. Biother. 2*:67-73 (1990)).

Technology to develop MAbs that can circumvent these particular problems has met with a number of obstacles. This is especially true for MAbs directed to human tumor antigens that have been developed for the diagnosis and treatment of cancer. Since many human tumor antigens are not recognized as foreign by the human immune system, they probably lack immunogenicity in man. In contrast, those human tumor antigens that are immunogenic in mice can be used to induce murine MAbs which specifically recognize the human antigen, and which may also have therapeutic utility in humans, but have the previously referred to limitations.

Proposals have therefore been made for making non-human MAbs less antigenic and more cytocidal in humans. Such techniques can be generically termed "humanization" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

In particular, one procedure which has been proposed for the preparation of humanized antibodies is the so-called chimerization procedure.

Such chimerization procedures involve production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Some early methods for carrying out such a chimerization procedure are described in EP-A-0120694 (Celltech Limited), EP-A-0125023

(Genentech Inc. and City of Hope), EP-A-01714906 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University), and EP-A-0194276 (Celltech Limited). The latter Celltech application also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin derived protein in place of the Fc portion of the human immunoglobulin.

Most human MAbs obtained *in vitro* are of the IgM class or isotype which makes them difficult to purify and limits their *in vivo* application (Shin, S.U. *Biother. 3*:43-53 (1991)). To obtain human MAbs of the IgG isotype, it has been necessary to use complex techniques (e.g. cell sorting) to first identify and then isolate those few cells producing IgG antibodies. A need therefore exists for an efficient way to switch antibody classes at will for any given antibody of a predetermined or desired antigenic specificity.

The present invention bridges both the hybridoma and genetic engineering technologies to provide a quick and efficient method, as well as products derived therefrom, for the production of a chimeric human/non-human antibody which recognizes the SF-25 antigen.

The chimeric antibodies of the present invention embody a combination of the advantageous characteristics of MAbs derived from mouse-mouse hybridomas and of human MAbs. The chimeric MAbs, like murine MAbs can recognize and bind to the human SF-25 antigen; however, unlike murine MAbs the species-specific properties of the chimeric antibodies will avoid the inducement of harmful hypersensitivity reactions and will allow for resistance to clearance when used in humans *in vivo*. Moreover, using the methods disclosed in the present invention, any desired antibody isotype can be conferred upon a particular antigen combining site. The chimeric antibodies of the present invention may be useful in the diagnosis and treatment of cancer which express the SF-25 antigen in humans and other animals.

B. Colorectal Cancer and Hepatic Metastases

Some tumors and other diseased tissues selectively express constitutive antigens which are not expressed by normal animal tissues. An example is colorectal adenocarcinoma and its SF-25 constitutive antigen.

Colorectal cancer is one of the most common malignancies in both men and women in the Western world. More than 150,000 new cases will be diagnosed in 1991 in the United States alone (Boring *et al.*, *Cancer Statistics 41*:19-36 (1991)). Despite major advances in general patient care and surgical therapy, the mortality rate associated with this disease has not changed significantly over the last forty years (Fleischer *et al.*, *JAMA 261*:580-586 (1989)). Indeed, about 60,000 patients die of this disease each year in this country principally because of advanced disease or recurrence (Cancer Facts & Figures - 1990, *American Cancer Society, Inc.*, Atlanta, Ga. (1990)).

Sixty percent of the patients with advanced colon adenocarcinoma will develop hepatic metastases (Weiss, L., *J. Pathol. 150*:195-203 (1986)). Although 70-80% of these patients will present with operable tumors at the time of diagnosis, even complete surgical resection is often unable to permit long term survival due to the presence of occult disease or hepatic micrometastases. Numerous post operative adjuvant treatment regimens have failed to reduce the incidences of hepatic metastases and tumor recurrence (Grem, J.L., *Semin. Oncol. 18 (Suppl. 1)*:17-26 (1991); Buyse *et al.*, *JAMA 259*:3571-3578 (1988); Mayer, R.J., *N. Engl. J. Med. 322*:399-401 (1990); Wolmark *et al.*, *J. Natl. Cancer Inst. 80*:30-36 (1988)). The death rate will undoubtedly remain the same until improved methods for the treatment of hepatic involvement become available. Thus, new, useful clinical treatment regimens are needed.

C. The SF-25 Antigen

A repertoire of MAbs against transformed cells of human endodermal origin has been developed (Wilson *et al.*, *Proc. Natl. Acad. Sci.* *85*:3140-3144 (1988)). One such murine MAb, SF-25, recognizes a 125 Kd cell surface glycoprotein designated the SF-25 antigen (Takahashi *et al.*, *Cancer Res.* *48*:6573-6579 (1988)). The hybridoma which secretes the SF-25 MAb was deposited under the provisions of the Budapest Treaty at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, and has been given the ATCC designation HB 9599.

Murine MAb SF-25 recognizes the SF-25 antigen which is highly expressed in human colon adenocarcinomas, their hepatic metastases, and other primary tumors of endodermal origin. Murine MAb SF-25 has been immunolocalized in tumor xenografts in nude mice (Takahashi *et al.*, *Cancer Res.* *48*:6573-6579 (1988); Takahashi *et al.*, *Gastroenterol.* *96*:1317-1329 (1989)). Furthermore, this antibody has been shown to be useful for the immunolocalization of colon adenocarcinoma *in vivo* (Id.).

Metabolic and cell surface labeling studies have demonstrated that the SF-25 antigen is a disulfide-bond-linked heterodimer which is composed of two glycosylated subunits termed α and β. The expression of the SF-25 antigen in colon adenocarcinoma tissues is uniform in contrast to the heterogeneous expression of other tumor associated antigens (Atkinson *et al.*, *Cancer Res.* *42*:4820-4823 (1982); Hand *et al.*, *Id.* *43*:728-735 (1983)). In addition, it appears that there is no antigenic modulation of the SF-25 antigen in liver metastases (Takahashi *et al.*, submitted to *Cancer Res.*). The SF-25 antigen and antibodies which recognize this antigen have been extensively described in related U.S. Patent Application Serial Numbers 07/203,198 and 07/130,777 which were filed on June 7, 1988 and December 9, 1987, respectively, the contents of which are herein incorporated by reference.

The SF-25 antigen is a constitutive antigen that is expressed on most if not all tumors of endodermal origin. The SF-25 antigen has been shown by

immunohistological staining to be expressed by the following human tumor types: colon adenocarcinoma; rectal adenocarcinoma; hepatocellular carcinoma; cholangiocellular carcinoma; gastric adenocarcinoma; breast adenocarcinoma; pancreatic adenocarcinoma; bladder adenocarcinoma; squamous cell carcinoma of the lung;adenocarcinoma of the lung; small cell carcinoma of the lung; large cell carcinoma of the lung; kidney carcinoma; ovary adenocarcinoma; cervix carcinoma; endometrial adenocarcinoma; choriocarcinoma; leukemia; lymphoma; and malignant melanoma. Previous studies of the SF-25 antigen's distribution revealed that a number of normal tissues were found to be negative by immunohistological staining including: esophagus; stomach; small intestine; colon; liver; bile duct; spleen; adrenal gland; lung; thyroid; skin; skeletal muscle; myocardium; connective tissue; brain; and spinal cord. Positive staining was present in a subpopulation of proximal tubular cells of the kidney. Weak staining was also observed in normal islet cells of the pancreas.

The SF-25 antigen is localized on the tumor cell surface and antibody binding to the SF-25 antigen does not induce internalization therefrom. The SF-25 antigen is not shed from the cell when it is examined by radioimmunoassay in culture supernatant and flow cytometric analysis (Takahashi *et al., Cancer Res. 48*:6573-6579 (1988)). Furthermore, MAb SF-25 has a high association constant (K_A = 1.36 x 10^8/M) and is able to immunolocalize to human colon adenocarcinomas established in the livers of nude mice (Takahashi *et al., Gastroenterology 96*:1317-1329 (1989)). The high number of antibody binding sites per cell (2.5 x 10^5/colon adenocarcinoma cell) suggest that the SF-25 MAb will be bound to the tumor cells in a high density. Taken together, these properties suggest that the SF-25 MAb may be effective as an immunotherapeutic reagent (Schlom *et al.*,in *Monoclonal Antibodies in Cancer: Advances in Diagnosis and Treatment*, (Roth, J.A. Ed.), Futura Publishing Company, Mount Kisco, NY, 1-65 (1986); Oldham, R.K., *in Biological Response Modifiers and Cancer Therapy*, (Chlao, J.K., Ed.) Marcel Dekker, Inc. New York, 3-16 (1988)).

D. Monoclonal Antibodies and the Treatment of Cancer Patients

Possible anti-tumor mechanisms mediated by MAbs include: 1) induction of tumor cytotoxicity by effector cells such as NK-cells and macrophages (Ravetch *et al.*, *Ann. Rev. Immunol.* 9:457-492 (1991)); 2) activation of complement and induction of complement-mediated cytotoxicity (Frank, M. M., *N. Engl. J. Med. 316*:1525-1530 (1987)); 3) interference with cell growth or differentiation by binding growth factors or receptors on the surface of tumor cells (Sporn *et al.*, *Nature 313*:745-747 (1985)); Rodeck *et al.*, *Cancer Res. 47*:3692-3696 (1987)); 4) induction of anti-idiotypic antibodies which subsequently have been used as novel vaccines against tumors (Wettendorff *et al.*, *Proc. Natl. Acad. Sci. USA 86*:3787-3791 (1989)); and 5) delivery of cytotoxic agents such as drugs, toxins, and radionucleotides to the tumor cells (Vitetta *et al.*, *Science 238*:1098-1104 (1987); Waldmann, T.A., *Science 252*:1657-1662 (1991); Dillman, R.O., *Ann. Int. Med. 111*:592-603 (1989)).

Cells with cytotoxic potential that bear receptors for the Fc fragment of IgG (FcγR) may bind and lyse target cells in the presence of antibody (antibody-dependent cell-mediated cytotoxicity; ADCC) (Kay *et al.*, *J. Immunol. 118*:2058-2066 (1977); Lubeck *et al.*, *Cell. Immunol. 111*:107-117 (1988)). ADCC requires the simultaneous binding of the Fab fragment of the antibody to its antigen and the binding of the Fc fragment to FcγR expressed on the effector cells. Macrophages express the three types of the FcγR which have been identified in human cells (FcγRI, II and III). FcγRI is found only on macrophages and is important for ADCC. NK-cells only express low affinity FcγR type III which will initiate ADCC by NK-cells upon binding to antibody (Ravetch *et al.*, *Ann. Rev. Immunol.* 9:457-492 (1991); Unkeless *et al.*, *Id.* 6:251-281 (1988); Adams *et al.*, *Id.* 2:283-318 (1984); Perussia *et al.*, *J. Exp. Med. 170*:73-86 (1989); Vivier *et al.*, *J. Immunol. 146*:206-210 (1991)).

The initial use of unmodified murine MAbs to treat humans with cancer has been disappointing. Only 23 partial and 3 complete remissions have been reported among 185 patients in 25 clinical trials. This is partially due to the fact that most mouse MAbs are not cytocidal against neoplastic cells in humans because these MAbs do not participate in human-complement or cell-mediated cytotoxicity (Waldmann, T.A., *Science 252*:1657-1662 (1991); Catane *et al.*, *J. Med. Sci. 24*:471 (1988)).

A chimeric MAb (c-SF-25 MAb) that has the Fc fragment of human IgG1 and the Fab fragment of the murine SF-25 MAb has been mentioned in Takahashi *et al.*,*Hepatology A12*:915 (1990); Takahashi, *et al. J. Cell. Biochem. Suppl. 15*:Part E pg. 139 (1991); Takahashi *et al.*, *Immunocon. Radiopharm. 4*:208 (1991); *Id.*, p. 237) (Takahashi *et al.*, *Antibody Immunocon. Radiopharm. 3*:86 (1990)). This chimeric construct induces ADCC by both human NK-cells and macrophages *in vitro*, since the FC fragment of human IgG1 interacts with FcγRI and also with FcγR type III.

IV. Summary of the Invention

The present invention provides a polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human immunoglobulin chain, both said sequences in operable linkage with each other.

The present invention also provides a host transformed with a polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human

immunoglobulin chain, both said sequences in operable linkage with each other.

The present invention also provides a eukaryotic or prokaryotic host transfected with a polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human immunoglobulin chain, both said sequences in operable linkage with each other.

The present invention also provides a chimeric immunoglobulin heavy chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

The present invention also provides a chimeric immunoglobulin light chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

The present invention also provides a chimeric antibody molecule comprising two light chains and two heavy chains, each of said chains comprise at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody given the ATCC designation HB 9599, or a fragment or derivative of said chimeric antibody.

The present invention also provides a process for preparing a chimeric immunoglobulin heavy chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599,comprising culturing a host capable

of expressing said heavy chain under culturing conditions; expressing said heavy chain; and recovering said heavy chain from said culture.

The present invention also provides a process for preparing a chimeric immunoglobulin light chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising culturing a host capable of expressing said light chain under culturing conditions; expressing said light chain; and recovering said light chain from said culture.

The present invention also provides a process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the hybridoma cell line given the ATCC designation HB 9599 comprising culturing a host capable of expressing said light chain under culturing conditions, expressing said light chain, and recovering said light chain from said culture; separately culturing a host capable of expressing said heavy chain under culturing conditions, expressing said heavy chain, and recovering said heavy chain from said culture; and associating said recovered heavy chain and light chain, thereby preparing said chimeric immunoglobulin, fragment or derivative.

The present invention also provides a process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising co-culturing a host capable of expressing said heavy chain with a host capable of expressing said light chain under culturing conditions; expressing said heavy chain and said light chain; permitting said heavy chain and said light chain to

associate into said chimeric immunoglobulin, fragment or derivative; and recovering said chimeric immunoglobulin, fragment or derivative from said culture.

The present invention also provides a process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the hybridoma cell line given the ATCC designation HB 9599, comprising culturing a host capable of expressing said heavy chain and said light chain under culturing conditions; expressing said chimeric immunoglobulin, fragment or derivative and recovering from said culture said chimeric immunoglobulin, fragment or derivative.

The present invention also provides an immunoassay method for detecting an antigen capable of binding to the chimeric SF-25 monoclonal antibody in a sample comprising contacting said sample with the detectably labeled antibody, fragment or derivative thereof; and detecting said antigen by detecting the binding of said antigen to said antibody, fragment or derivative.

The present invention also provides an imaging method for detecting a tissue antigen capable of binding to the chimeric SF-25 monoclonal antibody, comprising contacting the detectably labeled antibody, fragment or derivative thereof with said tissue; and detecting said antigen.

The present invention also provides a process for the manufacture of a pharmaceutical composition for use in the killing of cells expressing an antigen, which antigen is capable of binding to the chimeric SF-25 monoclonal antibody, comprising as an active ingredient an effective dose of the antibody, fragment or derivative thereof.

The present invention also provides a process for the manufacture of a pharmaceutical composition for use in treating an animal having a tumor expressing an antigen, which antigen is capable of binding to the chimeric SF-25 monoclonal antibody, comprising as an active ingredient an effective dose

of the antibody, fragment or derivative thereof. The pharmaceutical compositions of the present invention can be used to treat animals with the following tumors: colon adenocarcinoma; hepatocellular carcinoma; cholangiocellular carcinoma; gastric adenocarcinoma; rectal adenocarcinoma; breast adenocarcinoma; bladder adenocarcinoma; squamous cell carcinoma of the lungs; adenocarcinoma of the lungs; large cell carcinoma of the lungs; small cell carcinoma of the lungs; lymphoproliferative disease; myeloproliferative disease; lymphoma; leukemia; kidney carcinoma; ovary adenocarcinoma; cervical carcinoma; uterine endometrial adenocarcinoma; liver hepatoma; choriocarcinoma; malignant melanoma, as well as pancreatic adenocarcinoma and other pancreatic cancers. Previous studies of the SF-25 antigen's distribution revealed that a number of normal tissues were found to be negative by immunohistological staining including: esophagus; stomach; small intestine; colon; liver; bile duct; spleen; adrenal gland; lung; thyroid; skin; skeletal muscle; myocardium; connective tissue; brain; and spinal cord. Positive staining was present in a subpopulation of proximal tubular cells of the kidney. Weak staining was also observed in normal islet cells of the pancreas.

The present invention also provides for the use of an effective dose of an SF-25 antibody or fragment or derivative thereof, including a chimeric SF-25 monoconal antibody, for killing cells expressing an antigen, which antigen is capable of binding to said SF-25 antibody.

The present invention also provides for the use of an effective dose of an SF-25 antibody, or fragment or derivative thereof, including a chimeric SF-25 monoclonal antibody, for treating an animal having a tumor expressing an antigen, which antigen is capable of binding to said SF-25 antibody.

The present invention also provides for the use of an effective dose of an SF-25 antibody or fragment or derivative thereof, including a chimeric SF-25 monoclonal antibody, for treating an animal which has pancreatic carcinoma or other pancreatic cancer, which expresses an antigen, which antigen is capable of binding to said SF-25 antibody.

The present invention also provides for the use of an effective dose of an SF-25 antibody, or fragment or derivative thereof, including a chimeric SF-25 monoclonal antibody for treating an animal having cancer which expresses an antigen, which antigen is capable of binding to said SF-25 antibody, with the cancer selected from the group consisting of colon adenocarcinoma, hepatocellular carcinoma, cholangiocellular carcinoma, gastric adenocarcinoma, rectal adenocarcinoma, breast adenocarcinoma, bladder adenocarcinoma, squamous cell carcinoma of the lungs, adenocarcinoma of the lungs, large cell carcinoma of the lungs, small cell carcinoma of the lungs, lymphoproliferative disease, myeloproliferative disease, lymphoma, leukemia, kidney carcinoma, ovary adenocarcinoma, cervical carcinoma, uterine endometrial adenocarcinoma, liver hepatoma, choriocarcinoma, malignant meloma and pancreatic carcinoma, including the primary tumors or metastases or micrometastases of these diseases.

The present invention also provides a method of killing cells expressing an antigen, which antigen is capable of binding to an SF-25 antibody, including a chimeric SF-25 monoclonal antibody, comprising delivering to said cells an effective dose of the antibody, fragment or derivative thereof, and allowing said killing to occur.

The present invention also provides a method of treating an animal suspected of having a tumor expressing an antigen which is capable of binding to an SF-25 antibody, especially a chimeric SF-25 monoclonal antibody, comprising administering to said animal an effective dose of the antibody, fragment or derivative thereof.

The present invention also provides a method of treating a cancer in an animal which comprises administering an effective dose of an antibody specific

to the SF-25 antigen, especially a chimeric SF-25 antibody, or a derivative or fragment thereof, to said animal having a cancer selected from the group consisting of colon adenocarcinoma, hepatocellular carcinoma, cholangiocellular carcinoma, gastric adenocarcinoma, rectal adenocarcinoma, breast adenocarcinoma, bladder adenocarcinoma, squamous cell carcinoma of the lungs, adenocarcinoma of the lungs, large cell carcinoma of the lungs, small cell carcinoma of the lungs, lymphoproliferative disease, myeloproliferative disease, lymphoma, leukemia, kidney carcinoma, ovary adenocarcinoma, cervical carcinoma, uterine endometrial adenocarcinoma, liver hepatoma, choriocarcinoma, malignant meloma and pancreatic carcinoma, including the primary tumors or metastases or micrometastases of these diseases.

V. Brief Description of the Figures

Figure 1. Restriction maps of the germline J_K region and the 3.2 Kb HindIII fragment containing the functionally rearranged SF-25 V_K gene. The bracket above the germline restriction map indicates the J_K probe used (Figure 1A). Restriction maps of the germline J_H region and the 4.5 Kb EcoR1 fragment containing the functionally rearranged SF-25 V_H gene (Figure 1B). Exons are represented with solid boxes. The bracket above the germline restriction map indicates the J_H probe used. EcoR1 (E), HindIII (H), Xbal (X), and BamH1 (B) restriction enzyme sites are shown. "E" corresponds to the enhancer element present in the mouse H chain intron. "L" indicates the exon containing the leader peptide sequence. The L chain enhancer is provided by the L chain expression vector.

Figure 2. Structure of the SF-25 chimeric L and H chain expression vectors. The SF-25 L chain expression vector containing the xanthine-guanine phosphoribosyl (gpt) gene (Figure 2A). The SF-25 H chain expression vector containing the human Igγ1 constant region (Figure 2B).

Figure 3. Competitive inhibition of ^{125}I-labeled c-SF-25 MAb binding to LS 180 cells by unlabeled chimeric and murine SF-25 MAb. The binding of ^{125}I-labeled c-SF-25 MAb was inhibited by both chimeric and murine SF-25 MAb. In contrast, this binding was not inhibited by an unlabeled non-relevant MAb (B2TT). These results demonstrate that c-SF-25 MAb recognizes the same epitope as the murine MAb.

Figure 4. Antibody dependent cell-mediated cytotoxicity (ADCC) mediated by human effector cells in a 4 hour ^{51}Cr-release assay using LS 180 as the target cell. c-SF-25 MAb induced ADCC but the murine SF-25 MAb did not produce this activity by human PBLs (Figure 4A). Both purified NK-cells and macrophages mediated strong ADCC activity in the presence of c-SF-25 MAb (Figure 4B).

Figure 5. All control animals developed multiple "cannon ball-like" hepatic metastases of human colon adenocarcinomas (Figure 5A). A representative liver of an animal treated with c-SF-25 MAb which is free of detectable disease (Figure 5B). The $F(ab')_2$ fragment of c-SF-25 MAb had little effect on the degree of hepatic metastases (Figure 5C) and all mice developed hepatic tumors similar to controls.

Figure 6. ADCC mediated by murine effector cells in an 8 hour ^{51}Cr-release assay. Thioglycolate-elicited murine macrophages mediated ADCC against LS 180 cells in the presence of c-SF-25 MAb (Figure 6A). Murine splenocytes (Figure 6B) and murine NK-cells purified from these splenocytes (Figure 6C) also exhibited ADCC activity against LS 180 cells in the presence of c-SF-25 MAb.

Figure 7. Survival curves of mice treated with c-SF-25 MAb. All mice treated with c-SF-25 MAb survived more than six weeks, whereas all control mice died within six weeks after LS 180 cell injection. The survival rate of c-SF-25 MAb-treated animals was significantly longer than that of controls ($p < 0.0002$).

Figure 8. ADCC produced in the presence of c-SF-25 MAb (20 μg/ml) by murine splenocytes. Cytotoxicity was studied using LS 180 human colon adenocarcinoma cells at different effector to target (E:T) ratios.

Figure 9. Direct binding of c-SF-25 and MAb to LS 180 cells at different temperatures. The specific binding of ^{125}I-labeled c-SF-25 MAb increased as the temperature increased from 4°C to 37°C.

Figure 10. Direct binding of ^{125}I-labeled GA733 and 323/A3 MAbs to LS 180 tumor cells. GA733 and 323/A3 MAbs showed high binding to LS 180 cells both at 4°C and at 37°C.

Figure 11. ADCC mediated by murine NK-cells and macrophages at an E:T ratio of 50 to 1 in the presence of different concentrations of MAbs. ADCC activity by murine splenocytes was induced by c-SF-25 at all antibody concentrations tested (Figure 11A). Chimeric SF-25 MAb induced similar ADCC by macrophages (Figure 11B).

VI. Definitions

In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided. Any terms which are not specifically defined in this or other sections of this patent application have the ordinary meaning they would have when used by one of skill in the art to which this invention applies.

As used herein, an effective derivative or fragment of an antibody means a derivative or fragment of an antibody which is still capable of selectively binding to the same molecule(s) as that which the whole antibody binds to.

As used herein, a constitutive antigen means an antigen that is produced by the majority or all of the cells of a particular tumor type or disease type.

As used herein, imaging means the visualization or location of cells, or tumors, or other diseased tissues which express constitutive antigens and which bind detectably labeled, targeted, cytotoxic, effector cells to them.

As used herein, a detectable label is an atom or molecule which is attached to the targeted, cytotoxic, effector cell or constituent thereof, and which is used in imaging cells or tumors or other diseased tissues. Examples of such labels include, but are not limited to, radioisotopic labels, non-radioactive isotopic labels, chemiluminescent labels, fluorescent labels and enzyme labels.

VII. Description of the Preferred Embodiments

The present invention derives from the discovery of a chimerized SF-25 antibody with specificity for the human tumor SF-25 antigen which is constitutively expressed by human colon adenocarcinoma cells and other human tumors of endodermal origin. The present invention also derives from a method to produce these chimerized SF-25 antibodies and from methods to use them diagnostically and therapeutically.

In the present application, the term "chimeric antibody molecule" is used to describe an antibody molecule having heavy and/or light chains comprising at least the variable regions of heavy and/or light chains derived from one immunoglobulin molecule linked to at least part of a second protein. The second protein may comprise additional antibody constant region domains derived from a different immunoglobulin molecule or a non-immunoglobulin protein. The term "humanized chimeric antibody molecule" is used to describe a molecule having heavy and light chain variable region domains derived from an immunoglobulin from a non-human species, the remaining immunoglobulin constant region domains of the molecule being derived from a human immunoglobulin.

A. Production of the Chimeric SF-25 Monoclonal Antibody

1. SF-25 Antibodies and Antibody Fragments

In the following description, reference will be made to various methodologies well-known to those skilled in the art of immunology. Standard reference works setting forth the general principles of immunology include the work of Klein, J. (*Immunology: The Science of Cell-Noncell Discrimination*, John Wiley & Sons, New York (1982)); Kennett *et al.* (*Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses*, Plenum Press, New York (1980)); Campbell, A. ("Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon *et al.*, eds.), Elsevier, Amsterdam (1984)); and Eisen, H.N., (In: *Microbiology*, 3rd Ed. (Davis *et al.*, Harper & Row, Philadelphia (1980)).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. As used herein, the term "hapten" is intended to refer to any molecule capable of being bound by an antibody. The term "epitope" is meant to refer to that portion of a hapten which can be recognized and bound by an antibody. A hapten or antigen may have one, or more than one epitope. An "antigen" is a hapten which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. The specific reaction referred to above is meant to indicate that the hapten will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term "antibody" (Ab) or "monoclonal antibody" (Mab) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of binding a hapten. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody,

clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)).

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the SF-25 antigen can be administered to an animal in order to induce the production of sera containing polyclonal antibodies that are capable of binding the SF-25 antigen. In a preferred method, a preparation of SF-25 antigen is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or hapten binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al., Nature 256*:495 (1975); Kohler *et al., Eur. J. Immunol. 6*:511 (1976); Kohler *et al., Eur. J. Immunol. 6*:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with SF-25 antigen or, more preferably, with an SF-25-expressing cell. Although any such cell may be employed in accordance with the present invention, it is preferable to employ the hepatocellular carcinoma cell line, FOCUS (Lun *et al., In Vitro 20*:493-504 (1984)). Suitable cells can be recognized by their capacity to bind anti-SF-25 antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at 56°C), and supplemented with 10 μg/l of nonessential amino acids, 1,000 U/ml of penicillin, and 100 μg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP_2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybri-

doma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterology 80*:225-232 (1981), which reference is herein incorporated by reference). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the SF-25 antigen. A preferred hybridoma cell line, obtained by this process, is the monoclonal antibody-producing cell line "SF-25." This cell line produces monoclonal antibody "SF-25" which is capable of binding to the SF-25 antigen. Cell line "SF-25" was deposited under the provisions of the Budapest Treaty with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, on December 8, 1987, and given the ATCC designation: HB 9599.

Through application of the above-described methods, additional cell lines capable of producing antibodies which recognize epitopes of the SF-25 antigen can be obtained.

Alternatively, additional antibodies capable of binding to the SF-25 antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, antibodies capable of binding the SF-25 antigen are used to immunize an animal. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce antibody whose ability to bind to anti-SF-25 antibody can be specifically blocked by the SF-25 antigen. Such antibodies comprise anti-idiotypic antibodies to the anti-SF-25 antibody. Such antibodies can be used to immunize an animal, and thereby induce the formation of anti-SF-25 antibodies. Since anti-idiotypic antibodies can be used to immunize an animal and thus provoke the production of anti-SF-25 antibodies, they provide one method for inducing, or enhancing, an animal's immune response to colon cancer.

It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibody of the present invention may be used according to the methods

disclosed herein for the detection and treatment of colon adenocarcinoma in the same manner as intact antibody. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). Alternatively, hapten-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

2. Production of the Chimeric SF-25 MAb by Recombinant Technology

The identification of the amino acid sequence of the c-SF-25 MAb or fragment of derivatives thereof, permits these molecules to be produced through the application of recombinant DNA techniques. For example, an oligonucleotide can be constructed which is capable of encoding the c-SF-25 MAb or fragment or derivative thereof. Such an oligonucleotide can be operably linked into an expression vector and introduced into a host cell to enable the expression of the c-SF-25 MAb or fragment or derivative thereof by that cell. Techniques for synthesizing such oligonucleotides are disclosed by, for example, Wu *et al.*, *Prog. Nucl. Acid. Res. Molec. Biol. 21*:101-141 (1978)). Procedures for constructing recombinant molecules in accordance with the above-described method are disclosed by Maniatis *et al.*, In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1984), and also in *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press, (Sambrook *et al.*, Eds.) Cold Spring Harbor, NY (1989) which references are herein incorporated by reference.

The identification of the amino acid sequence of the c-SF-25 MAb, or fragments of this MAb, also permits the cloning of the gene which encodes the c-SF-25 MAb.

Any of a variety of methods may be used to clone the c-SF-25 MAb gene. One such method entails analyzing a shuttle vector library of cDNA inserts (derived from an c-SF-25 MAb expressing cell) for the presence of an insert which contains the c-SF-25 MAb gene. Such an analysis may be

conducted by transfecting cells with the vector, and then assaying for c-SF-25 MAb expression. A preferred method for cloning the c-SF-25 MAb gene entails determining the amino acid sequence of the c-SF-25 MAb molecule. Although it is possible to determine the entire amino acid sequence of the c-SF-25 MAb molecule it is preferable to determine the sequence of peptide fragments of the molecule. If the peptides are greater than 10 amino acids long, this sequence information is generally sufficient to permit one to clone a gene such as the gene for the c-SF-25 MAb molecule.

To accomplish this task, c-SF-25 MAb molecules are preferably purified from producer cells by monoclonal antibody affinity chromatography and isolated by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis ("SDS-PAGE") and electroelution. The c-SF-25 MAb molecules are fragmented as with cyanogen bromide, or with proteases such as papain, chymotrypsin, trypsin, etc. (Oike *et al., J. Biol. Chem. 257*:9751-9758 (1982); Liu *et al., Int. J. Pept. Protein Res. 21*:209-215 (1983)). The resulting peptides are separated, preferably by reverse-phase HPLC, and subjected to amino acid sequencing. To accomplish this task, the protein is preferably analyzed by automated sequencers.

Once one or more suitable peptide fragments have been sequenced, the DNA sequences capable of encoding them are examined. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid (Watson, J.D., In: *Molecular Biology of the Gene*, 3rd Ed., W.A. Benjamin, Inc., Menlo Park, CA (1977), pp. 356-357). Using the genetic code, one or more different oligonucleotides can be identified, each of which would be capable of encoding the c-SF-25 MAb peptides. The probability that a particular oligonucleotide will, in fact, constitute the actual c-SF-25 MAb-encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic cells. Such "codon usage rules" are disclosed by Lathe *et al., J. Molec. Biol. 183*:1-12 (1985). Using the "codon usage rules" of Lathe, a single oligonucleotide, or a set of

oligonucleotides, that contains a theoretical "most probable" nucleotide sequence capable of encoding the c-SF-25 MAb peptide sequences is identified.

Although occasionally an amino acid sequence may be encoded by only a single oligonucleotide, frequently the amino acid sequence may be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the members of this set contain oligonucleotides which are capable of encoding the peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is identical to the nucleotide sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the peptide.

The oligonucleotide, or set of oligonucleotides, containing the theoretical "most probable" sequence capable of encoding the SF-25 MAb fragment is used to identify the sequence of a complementary oligonucleotide or set of oligonucleotides which is capable of hybridizing to the "most probable" sequence, or set of sequences. An oligonucleotide containing such a complementary sequence can be employed as a probe to identify and isolate the c-SF-25 MAb gene (Maniatis *et al., Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982).

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the c-SF-25 MAb gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified (using the above-described procedure), synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing the SF-25 gene. Single stranded oligonucleotide molecules complementary to the "most probable" SF-25 peptide encoding sequences can be synthesized using procedures which are

well known to those of ordinary skill in the art (Belagaje *et al., J. Biol. Chem. 254*:5765-5780 (1979); Maniatis *et al.*, In: *Molecular Mechanisms in the Control of Gene Expression*, Nierlich *et al.*, Eds., Acad. Press, NY (1976); Wu *et al., Prog. Nucl. Acid Res. Molec. Biol. 21*:101-141 (1978); Khorana, R.G., *Science 203*:614-625 (1979)). Additionally, DNA synthesis may be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Maniatis *et al.* (In: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1982)) and by Haymes *et al.* (In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)), which references are herein incorporated by reference. The source of DNA or cDNA used will preferably have been enriched for SF-25 MAb sequences. Such enrichment can most easily be obtained from cDNA obtained by extracting RNA from cells, such as hybridoma cells, which produce high levels of SF-25. An example of such a cell is the hybridoma cell line given the ATCC designation HB 9599 which was previously described.

To identify and clone the gene which encodes the SF-25 protein, a DNA, or more preferably a cDNA, library is screened for its ability to hybridize with the oligonucleotide probes described above. Suitable DNA preparations (such as human genomic DNA) are enzymatically cleaved, or randomly sheared, and ligated into recombinant vectors. The ability of these recombinant vectors to hybridize to the above-described oligonucleotide probes is then measured. Vectors found capable of such hybridization are then analyzed to determine the extent and nature of the SF-25 sequences which they contain. Based purely on statistical considerations, a gene such as that which encodes the c-SF-25 molecule could be unambiguously identified (via hybridization screening) using an oligonucleotide probe having only 18 nucleotides.

Thus, in summary, the actual identification of c-SF-25 peptide sequences permits the identification of a theoretical "most probable" DNA sequence, or a set of such sequences, capable of encoding such a peptide. By

constructing an oligonucleotide complementary to this theoretical sequence (or by constructing a set of oligonucleotides complementary to the set of "most probable" oligonucleotides), one obtains a DNA molecule (or set of DNA molecules), capable of functioning as a probe to identify and isolate the c-SF-25 MAb gene.

Techniques such as, or similar to, those described above have successfully enabled the cloning of genes for human aldehyde dehydrogenases (Hsu *et al., Proc. Natl. Acad. Sci. USA 82*:3771-3775 (1985)), fibronectin (Suzuki *et al., Eur. Mol. Biol. Organ. J. 4*:2519-2524 (1985)), the human estrogen receptor gene (Walter *et al., Proc. Natl. Acad. Sci. USA 82*:7889-7893 (1985)), tissue-type plasminogen activator (Pennica *et al., Nature 301*:214-221 (1983)) and human term placental alkaline phosphatase complementary DNA (Kam *et al., Proc. Natl. Acad. Sci. USA 82*:8715-8719 (1985)).

In a alternative way of cloning the c-SF-25 MAb gene, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing MAb c-SF-25) into an expression vector. The library is then screened for members capable of expressing a protein which binds to SF-25 antigen, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as MAb c-SF-25, or fragments or derivatives thereof. In this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing c-SF-25 MAb. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment.

An "expression vector" is a vector which (due to the presence of appropriate transcriptional and/or translational control sequences) is capable of expressing a DNA (or cDNA) molecule which has been cloned into the

vector and of thereby producing a polypeptide or protein. Expression of the cloned sequences occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Similarly, if a eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequences. Importantly, since eukaryotic DNA may contain intervening sequences, and since such sequences cannot be correctly processed in prokaryotic cells, it is preferable to employ cDNA from a cell which is capable of expressing c-SF-25 in order to produce a prokaryotic genomic expression vector library. Procedures for preparing cDNA and for producing a genomic library are disclosed by Maniatis *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982)).

The above-described expression vector genomic library is used to create a bank of host cells (each of which contains one member of the library). The expression vector may be introduced into the host cell by any of a variety of means (i.e., transformation, transfection, protoplast fusion, electroporation, etc.). The bank of expression vector-containing cells is clonally propagated, and its members are individually assayed (using an immunoassay) to determine whether they produce a protein capable of binding to SF-25 antigen.

The expression vectors of those cells which produce a protein capable of binding to SF-25 antigen are then further analyzed to determine whether they express (and thus contain) the entire c-SF-25 MAb gene, whether they express (and contain) only a fragment of the c-SF-25 MAb gene, or whether they express (and contain) a gene whose product, though immunologically related to c-SF-25 MAb, is not c-SF-25 MAb. Although such an analysis may be performed by any convenient means, it is preferable to determine the nucleotide sequence of the DNA or cDNA fragment which had been cloned into the expression vector. Such nucleotide sequences are then examined to

determine whether they are capable of encoding polypeptides having the same amino acid sequence as digestion fragments of SF-25.

An expression vector which contains a DNA or cDNA molecule which encodes the c-SF-25 MAb gene may, thus, be recognized by: (i) the ability to direct the expression of a protein which is capable of binding to SF-25 antigen; and (ii) the presence of a nucleotide sequence which is capable of encoding each of the fragments of c-SF-25 MAb. The cloned DNA molecule of such an expression vector may be removed from the expression vector and isolated in pure form.

Isolation of genomic DNA fragments containing the SF-25 light and heavy chain antibody variable region gene segments is also a method that may be used to clone the chimeric SF-25 MAb. In this method high molecular weight genomic DNA is isolated from the murine SF-25 cell line by standard techniques described in Maniatis *et al. supra*. This DNA can then be reduced to a smaller size by digestion with various restriction enzymes, by sonicating, by shearing by the application of mechanical force, or by a variety of other procedures. These genomic DNA fragments can then be ligated into various types of phage cloning vectors such as λgtWES, λgt10, or EMBL3 or plasmid cloning vectors such as pBR322, pUC18, or cosmid cloning vectors such as pHC79 and introduced into host bacterial. Other vectors and host organisms may be used to clone genomic SF-25 DNA fragments.

A collection of these genomic DNA fragments is referred to as a genomic DNA library. Once a library is constructed from the SF-25 genomic DNA, a variety of methods may be used to identify and/or isolate the genomic DNA fragments containing the SF-25 light and heavy chain antibody variable region gene segments. As described above, oligonucleotides capable of encoding a fragment of the SF-25 Mab gene may be labeled and used as a hybridization probe to isolate the genomic DNA fragment containing the SF-25 light or heavy chain variable region. Other nucleic acid probes that contain the mouse antibody constant region, the mouse J region, surrounding or flanking sequences, or sequences that corresponding to the SF-25 light and

heavy chain antibody variable region sequences such as a cDNA may be used as a hybridization probe.

It is contemplated that further "humanization" of the monoclonal antibodies of the invention may be accomplished by forming "mosaic" antibodies in which human sequences are also inserted into the variable region. For example, the variable regions of both mouse and human antibodies comprise four framework residues (FRs). Within the FRs are three complementarity determining residues (CDRs) which are responsible for antigen binding. A human-mouse mosaic having the desired binding characteristics may be made by inserting mouse CDR sequences within human framework residues. Such mosaic variants are contemplated equivalents of the chimeric immunoglobulins of the invention, as are partial chimeric immunoglobulins, e.g., in which only the heavy chain constant region of murine origin has been replaced by an equivalent sequence of human origin, or variants wherein one or more amino acids have been changed by directed mutagenesis.

3. Expression of the Cloned Chimeric SF-25 Gene

The present invention therefore provides a means for obtaining a DNA molecule which encodes the c-SF-25 MAb molecule. By operably linking this DNA molecule (or a fragment or mutated form of this DNA molecule) to a functional promoter, it is possible to direct the expression of the SF-25 MAb gene (or a fragment or derivative thereof) in a cell, or organism.

The expression of a DNA sequence requires that the DNA sequence be "operably linked" to DNA sequences which contain transcriptional and translational regulatory information. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, but shall in general include a promoter region which, in

prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal the initiation of protein synthesis. Regulatory regions in eukaryotic cells will in general include a promoter region sufficient to direct the initiation of RNA synthesis.

Two DNA sequences (such as a promoter region sequence and a c-SF-25 MAb-encoding sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of the c-SF-25 MAb-encoding sequence, or (3) interfere with the ability of the c-SF-25-MAb encoding sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of transcribing that DNA sequence.

To express the c-SF-25 MAb molecule (or a functional derivative thereof) in a prokaryotic cell (such as, for example, *E. coli*, *B. subtilis*, *Pseudomonas*, *Streptomyces*, etc.), it is necessary to operably link the c-SF-25 MAb-encoding sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ, the bla promoter of the β-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pPR325, etc. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the trp, recA, lacZ, lacI, and gal promoters of *E. coli* the α-amylase (Ulmanen *et al.*, *J. Bacteriol. 162*:176-182 (1985)) and the σ-28-specific promoters of B. subtilis (Gilman *et al.*, *Gene 32*:11-20 (1984)), the promoters of the bacteriophages of Bacillus (Gryczan In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward *et al.*, *Mol. Gen. Genet. 203*:468-478 (1986)). Prokaryotic promoters are reviewed by Glick, B.R., (*J. Ind. Microbiol. 1*:277-282 (1987)); Cenatiempo, Y.

(*Biochimie 68*:505-516 (1986)); and Gottesman, S. (*Ann. Rev. Genet. 18*:415-442 (1984)).

Proper expression in a prokaryotic cell requires the presence of a ribosome binding site upstream of the gene-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold *et al.* (*Ann. Rev. Microbiol. 35*:365-404 (1981)).

If expression is desired in a eukaryotic cell, such as yeast, fungi, mammalian cells, or plant cells, then it shall be necessary to employ a promoter capable of directing transcription in such a eukaryotic host. Preferred eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer *et al., J. Mol. Appl. Gen. 1*:273-288 (1982)); the TK promoter of Herpes virus (McKnight, S., *Cell 31*:355-365 (1982)); the SV40 early promoter (Benoist *et al., Nature (London) 290*:304-310 (1981)); and the yeast gal4 gene promoter (Johnston *et al., Proc. Natl. Acad. Sci. (USA) 79*:6971-6975 (1982); Silver *et al., Proc. Natl. Acad. Sci. (USA) 81*:5951-5955 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine of an oligonucleotide. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and an oligonucleotide which encodes the c-SF-25 MAb molecule (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the c-SF-25 MAb-encoding oligonucleotide) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the c-SF-25 MAb gene sequence).

An oligonucleotide which encodes the c-SF-25 MAb (or a functional derivative thereof) when operably linked to a functional promoter is preferably introduced into a recipient cell by any of a variety of suitable means: transformation, transfection, conjugation, protoplast fusion, electroporation, etc.

The c-SF-25 MAb-encoding sequence and an operably linked promoter may be introduced into a recipient cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the c-SF-25 MAb may occur through the transient expression of the introduced gene sequence. Alternatively, permanent expression may occur through the integration of the introduced gene sequence into the host chromosome.

Preferably, the introduced gene sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in E. coli (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX. Such plasmids are, for example, disclosed by Maniatis *et al.* (In: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1982)). *Bacillus* plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, T. (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307-329). Suitable *Streptomyces* plasmids include pIJ101 (Kendall *et al., J. Bacteriol. 169*:4177-4183 (1987)), and streptomyces bacteriophages such as ϕC31 (Chater *et al.*, In: *Sixth International Symposium on Actinomycetales Biology*, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). *Pseudomonas* plasmids are reviewed by John *et al.* (*Rev. Infect. Dis. 8*:693-704 (1986)), and Izaki, K. (*Jpn. J. Bacteriol. 33*:729-742 (1978)).

Preferred eukaryotic plasmids include BPV, vaccinia, SV40, 2-micron circle, etc. Such plasmids are well known in the art (Botstein *et al., Miami*

Wntr. Symp. 19:265-274 (1982); Broach, J.R., In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, J.R., *Cell 28*:203-204 (1982); Bollon *et al.*, *J. Clin. Hematol. Oncol. 10*:39-48 (1980); Maniatis, T., In: *Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Expression*, Academic Press, NY, pp. 563-608 (1980)).

A method of production of c-SF-25 may combine five elements:

1. Isolation of messenger RNA (mRNA) from the mouse hybridoma cell line which produces the SF-25 MAb, cloning and cDNA production therefrom;

2. Preparation of a full length cDNA library from purified mRNA from which the appropriate V region gene segments of the L and H chain genes can be: (i) identified with appropriate probes, (ii) sequenced, and (iii) made compatible with a C gene segment.

3. Preparation of C region gene segment modules by cDNA preparation and cloning.

4. Construction of complete H or L chain-coding sequences by linkage of the cloned specific immunoglobulin V region gene segments described in 2 above to cloned human C region gene segment modules described in 3.

5. Expression and production of chimeric L and H chains in selected hosts, including prokaryotic and eukaryotic cells.

One common feature of all immunoglobulin L and H chain genes and the encoded messenger RNAs is the so-called J region. H and L chain J regions (J_H and J_L) have different sequences, but a high degree of sequence homology exists (greater than 80%) among each group, especially near the C region. This homology is exploited in this invention wherein consensus sequences of J_H and J_L were used to design oligonucleotides for use as primers or probes for introducing useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments.

C region cDNA module vectors prepared from human cells and modified by site-directed mutagenesis to place a restriction site at the analogous position in the human sequence were used. For example, one can clone the complete human κ chain C region (C_κ) and the complete human gamma 1 C region ($C_\gamma 1$). An alternative method utilizing genomic C region clones as the source for C region module vectors would not allow these genes to be expressed in hosts such as bacteria where enzymes needed to remove intervening sequences are absent.

Cloned V region segments are excised and ligated to C_L or C_H module vectors. In addition, the human gamma 1 region can be modified by introducing a termination codon thereby generating a gene sequence which encodes the H chain portion of an Fab molecule.

The coding sequences with linked V and C regions are then transferred into appropriate expression vehicles for expression in appropriate hosts, prokaryotic or eukaryotic.

Two coding DNA sequences are said to be "operably linked" if the linkage results in a continuously translatable sequence without alteration or interruption of the triplet reading frame. A DNA coding sequence is operably linked to a gene expression element if the linkage results in the proper function of that gene expression element to result in expression of the coding sequence.

Expression vehicles include plasmids or other vectors. Preferred among these are vehicles carrying a functionally complete human C_H or C_L chain sequence having appropriate restriction sites engineered so that any V_H or V_L chain sequence with appropriate cohesive ends can be easily inserted. Human C_H or C_L chain sequence-containing vehicles are thus an important embodiment of the invention. These vehicles can be used as intermediates for the expression of any desired complete H or L chain in any appropriate host.

One preferred host is yeast. Yeast provides substantial advantages for the production of immunoglobulin L and H chains. Yeast carry out post-translational peptide modifications including glycosylation. A number of

recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for the production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e. prepeptides) (Hitzman *et al.*, *11th International Conference on Yeast, Genetics and Molecular Biology*, Montpellier, France, September 13-17, 1982).

Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of chimeric H and L chain proteins and assembled chimeric antibodies. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the iso-1-cytochrome C (CYC-1) gene can be utilized. A number of approaches may be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast.

Bacterial strains may also be utilized as transformation hosts for the production of antibody molecules or antibody fragments described by this invention. *E. coli* strains such as *E. coli* W3110 (ATCC 27325) and other enterobacteria such as *Salmonella typhimurium* or *Serratia marcescens*, and various *Pseudomonas* species may be used.

Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches may be taken for evaluating the expression plasmids for the production of chimeric antibodies or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria.

Other preferred hosts are mammalian cells, grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of H and L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein.

Mammalian cells which may be useful as hosts for the production of antibody proteins include cells of lymphoid origin, such as the hybridoma Sp2/O-Ag14 (ATCC CRL 1581) or the myeloma P3X63Ag8 (ATCC TIB 9), and their derivatives. Others include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO- K1 (ATCC CRL 61).

Many vector systems are available for the expression of cloned H chain and L chain genes in mammalian cells. Different approaches can be followed to obtain complete H_2L_2 antibodies. It is possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H_2L_2 antibodies. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both H and L chains can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells may be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with a H chain plasmid containing a second selectable marker. Cell lines producing H_2L_2 molecules via either route could be transfected with plasmids encoding additional copies of H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled (H_2L_2) antibody molecules or enhanced stability of the transfected cell lines.

B. Polypeptide Products

The present invention provides "chimeric" immunoglobulin chains, either H or L, with specificity toward human tumor cell SF-25 antigen. A

chimeric chain contains a C region substantially similar to that present in a natural human immunoglobulin, and a V region having the desired anti-tumor specificity of the invention.

The invention also provides immunoglobulin molecules having H and L chains associated so that the overall molecule exhibits the desired binding and recognition properties. Various types of immunoglobulin molecules are provided: monovalent, divalent, or molecules with the invention's V binding domains attached to moieties carrying desired functions.

This invention also provides for "fragments" of chimeric immunoglobulin molecules, which include Fab, Fab', and $F(ab')_2$ molecules. The invention also provides for "derivatives" of the chimeric immunoglobulins, which term includes those proteins encoded by truncated or modified genes to yield molecular species functionally resembling the immunoglobulin fragments. The modifications include, but are not limited to, addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins and tumor necrosis factor (TNF). The fragments and derivatives can be produced from any of the hosts of this invention.

Antibodies, fragments or derivatives having chimeric H chains and L chains of the same or different V region binding specificity, can be prepared by appropriate association of the individual polypeptide chains, as taught, for example by Sears *et al.* (*Proc. Natl. Acad. Sci. USA* 72:353-357 (1975)). With this approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

C. Diagnostic Uses of the Chimeric SF-25 Monoclonal Antibody

The c-SF-25 MAbs of the present invention are particularly suited for *in vivo* and *in vitro* imaging of certain tumors and other diseased tissues.

1. Labels

These chimeric antibodies, or fragments or derivatives thereof, may be labeled using any of a variety of labels and methods of labeling. Examples of types of labels which can be used in the present invention include, but are not limited to, enzyme labels, radioisotopic labels, non-radioactive isotopic labels, fluorescent labels, toxin labels, chemiluminescent labels, and nuclear magnetic resonance contrasting agents.

Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholine esterase, etc.

Examples of suitable radioisotopic labels include ^{3}H, ^{111}In, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{57}To, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, ^{90}Y, ^{67}Cu, ^{217}Ci, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd, etc. ^{111}In is a preferred isotope. Its use may have substantial advantages since its avoids the problem of dehalogenation of the ^{125}I or ^{131}I-labeled monoclonal antibody by the liver. In addition, this radionucleotide has a more favorable gamma emission energy for imaging (Perkins *et al., Eur. J. Nucl. Med. 10*:296-301 (1985); Carasquillo *et al., J. Nucl. Med. 28*:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhanced specificity of tumor localization (Esteban *et al., J. Nucl. Med. 28*:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Tr, ^{56}Fe, etc.

Examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a fluorescamine label, etc.

Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin, etc.

Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, Iron, etc.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to these chimeric antibodies, derivatives, or fragments thereof, can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy *et al.* (*Clin. Chim. Acta 70*:1-31 (1976)), and Schurs *et al.* (*Clin. Chim. Acta 81*:1-40 (1977)). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

2. *In vivo* Imaging Using the c-SF-25 MAb

The detection of cells which express the SF-25 antigen may be accomplished by the use of *in vivo* imaging techniques in which the labeled chimeric MAbs of the present invention, or fragments or derivatives thereof, are administered to a patient or other animal, and the presence of tumors or other diseased tissues expressing the antigen is detected without the prior removal of any tissue sample. Such *in vivo* detection procedures have the

advantage of being less invasive than other detection methods, and are, moreover, capable of detecting the presence of antigen-expressing cells in tissue which cannot be easily removed from the patient.

3. *In vitro* Imaging Using the c-SF-25 MAb

The chimerized antibodies or fragments or derivatives of the present invention are also particularly suited for use *in vitro* to detect cells which express the SF-25 antigen in body tissue, fluids (such as blood, lymph, etc.), stools, or cellular extracts.

The detection of cells which express the SF-25 antigen may be accomplished by removing a sample of tissue from a patient or other animal and then treating the isolated sample with any of the suitably labeled chimeric SF-25 antibodies of the present invention. Preferably, such *in vitro* detection is accomplished by removing a histological specimen from a patient or other animal, and providing the labeled chimeric antibodies of the present invention to such specimen by applying them or by overlaying them onto a sample of tissue. Through the use of such a procedure, it is possible to determine not only the presence of the SF-25 antigen, but also the distribution of the antigen on the examined tissue. Using the present invention, those of ordinary skill in the art will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in vitro* detection.

The c-SF-25 MAbs of the present invention may be used to quantitatively or qualitatively detect the presence of cells which express the SF-25 antigen. Thus the c-SF-25 MAb of the present invention may be employed to detect or visualize, by biopsy and histology, cancers or other diseased tissues which express the SF-25 antigen. Such detection may be accomplished using any of a variety of methods. For example, by radioactively labeling the cells of the present invention, it is possible to detect the targeted antigen through the use of radioimmune assays. A good

description of a radioimmune assay (RIA) may be found in *Laboratory Techniques and Biochemistry in Molecular Biology* by Work *et al.* (North Holland Publishing Company, NY (1978)), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein.

4. Use of c-SF-25 MAb in Immunoassays

The c-SF-25-MAbs, or fragments or derivatives thereof, of the present invention are particularly suited for use in immunodiagnostic assays for the SF-25 antigen wherein they may be utilized in a liquid phase or bound to a solid phase carrier and may be provided in kits.

These chimeric antibodies, or fragments or derivatives thereof, may be labeled using any of a variety of labels and methods of labeling. Examples of types of labels which can be used in the present invention include, but are not limited to, enzyme labels, radioisotopic labels, non-radioactive isotopic labels, fluorescent labels, toxin labels, and chemiluminescent labels, as described previously.

The detection of SF-25 antigens by the chimeric SF-25 antibodies, or fragments or derivatives thereof, of the present invention can be improved through the use of carriers. Well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to the SF-25 antigen. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Those skilled in the art will note many other suitable carriers for

binding monoclonal antibody, or will be able to ascertain the same by use of routine experimentation.

The chimeric SF-25 antibodies, or fragments or derivatives thereof, of the present invention may be used to quantitatively or qualitatively detect the presence of cells which express the SF-25 antigen. Such detection may be accomplished using any of a variety of immunoassays. For example, by radioactively labeling these chimeric derivatives or fragments thereof, it is possible to detect the SF-25 antigen through the use of radioimmune assays as previously described.

The c-SF-25 MAbs, derivatives or fragments thereof, of the present invention may also be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In such an immunometric assay, a quantity of unlabeled SF-25 MAbs or c-SF-25 MAbs is bound to a solid support that is insoluble in the fluid being tested (*i.e.*, blood, lymph, liquified stools, tissue homogenate, etc.) and a quantity of detectably labeled, c-SF-25 MAb or SF-25 MAbs is added to permit detection and/or quantitation of the ternary complex formed between solid-phase unlabeled antibody, the SF-25 antigen and the labeled antibody.

Typical immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the SF-25 antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted SF-25 antigen, if any, and then is contacted with the solution containing a known quantity of labeled c-SF-25 antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the SF-25 antigen bound to the solid support through the unlabeled antibody, the solid support is washed a second time to remove the unreacted labeled antibody. This type of forward sandwich assay may be a simple "yes/no" assay to determine whether SF-25 antigen is present or may be made quantitative by comparing the measure of labeled c-SF-25 antibody

with that obtained for a standard sample containing known quantities of SF-25 antigen. Such "two-site" or "sandwich" assays are described by Wide at pages 199-206 of *Radioimmune Assay Method*, edited by Kirkham and Hunter, E. & S. Livingstone, Edinburgh, 1970.

In another type of "sandwich" assay, which may also be useful with the antibodies of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the c-SF-25 antibody bound to the solid support and labeled c-SF-25 antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled c-SF-25 antibody. The presence of labeled c-SF-25 antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled c-SF-25 antibody to the fluid sample followed by the addition of unlabeled c-SF-25 antibody bound to a solid support after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the the residue of the sample being tested and the solution of unreacted, labeled c-SF-25 antibody. The determination of labeled c-SF-25 antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

As explained above, the immunometric assays for SF-25 antigen require that the particular binding molecule be labeled with a "reporter molecule." These reporter molecules or labels, as identified above, are conventional and well-known to the art. In the practice of the present invention, enzyme labels are a preferred embodiment. No single enzyme is ideal for use a label in every conceivable immunometric assay. Instead, one must determine which enzyme is suitable for a particular assay system. Criteria important for the choice of enzymes are turnover number of the pure enzyme (the numer of substrate molecules converted to produce per enzyme site per unit of time), purity of the enzyme preparation, sensitivity of detection

of its product, ease and speed of detection of the enzyme reaction, absence of interfering factors or of enzyme-like activity in the test fluid, stability of the enzyme and its conjugate, and the like. Included among the enzymes used as preferred labels in the immunometric assays of the present invention are peroxidase, alkaline phosphatase, beta-galactosidase, urease, glucose oxidase, glycoamylase, malate dehydrogenase, and glucose-6-phosphate dehydrogenase. Urease is among the more preferred enzyme labels, particularly because of chromogenic pH indicators which make its activity readily visible to the naked eye.

The above-described *in vitro* or *in vivo* detection methods may be used in the diagnosis of certain cancers, such as colon adenocarcinoma, or other cancers and diseases which express the SF-25 antigen. Additionally, such detection methods may be used to assist in the determination of the stage of a malignancy or other disease, or to determine whether an individual possesses malignant or other lesions which may be obscured (or whose detection may be complicated) by the close association of normal tissue.

One especially preferred use for the c-SF-25 MAbs of the present invention is as an aid in the diagnosis of colon cancer in patients who present with symptoms of inflammatory bowel diseases, and in particular, ulcerative colitis or intestinal polyps. Using the methods of the prior art, the early diagnosis and detection of colon cancer in individuals suffering from such inflammatory bowel disease is often complicated, or masked, by the symptoms of bowel disease. Thus, concern that an occult colorectal carcinoma may be present in an individual suffering from inflammatory bowel disease may result in a recommendation that such individuals submit to a colectomy. Because the c-SF-25 MAbs of the present invention are capable of identifying colorectal carcinomas, they can be used to determine the presence of otherwise occult lesions. Thus, their use in the diagnosis of the cause and severity of inflammatory bowel disease and colorectal carcinoma is capable of preventing unwarranted colectomies, and is, therefore, highly desirable.

As used herein, an effective amount of c-SF-25 MAbs is one capable of achieving the desired diagnostic discrimination. The amount of c-SF-25 MAb which is typically used in a diagnostic test is generally between 1-10 mg, and preferably between 1μg to 1mg.

D. Therapeutic Uses of the Present Invention

In addition to providing methods for diagnosing and treating colon cancer, pancreatic andenocarcinoma, or other cancers of endodermal origin, the c-SF-25 MAbs of the present invention also provide a means for preventing the onset of these cancers, and for treating affected animals including humans. The discovery that the SF-25 antigen is constitutively expressed on colon cancer cells, and the identification of c-SF-25 MAbs capable of binding to this antigen provide a means for preventing and treating these cancers and other diseased tissues which express the SF-25 antigen.

The antibodies of this invention can be adapted for therapeutic efficacy by virtue of their ability to mediate ADCC and/or complement-dependent cytotoxicity (CDC) against tumor cells. For these activities, either an endogenous source or an exogenous source of effector cells (for ADCC) or complement components (for CDC) can be utilized.

The chimeric antibodies, fragments or derivatives of this invention may be advantageously utilized in combination with other chimeric antibodies, or with lymphokines or hemopoietic growth factors, etc., which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention having a complete or partial human C region can be introduced *in vivo*, especially in humans, with reduced negative immune reactions such as serum sickness or anaphylactic shock, as compared to whole mouse antibodies.

The ability to conjugate the chimeric SF-25 antibodies, or fragments or derivatives thereof, with toxin molecules provides an additional method for treating colon cancer and other cancers and diseased tissues which

constitutively express the SF-25 antigen. In this embodiment, the c-SF-25 MAb, or derivatives or fragments of these antibodies, which are capable of recognizing the SF-25 antigen, are conjugated with toxin molecules and administered to a patient suspected of having colon or other cancer. When such a toxin derivatized MAb binds to a colon or cancer cell, the toxin moiety will cause the death of the cancer cell.

Any of a variety of toxin molecules may be employed to produce such toxin-conjugated, c-SF-25 MAbs. Examples of suitable cytotoxic molecules include: ricin; diphtheria, pseudomonas, and cholera toxins; TNF, etc. Toxins conjugated to antibodies or other ligands are known in the art (see, for example, Olsnes *et al.*, *Immunol. Today 10*:291-295 (1989)).

Additional types of therapeutic moieties including, but not limited to, radionuclides and cytotoxic drugs and other agents, can be conjugated to the c-SF-25 MAbs of the present invention to treat cancer patients. Examples of radionuclides which can be coupled to the cells of the present invention and delivered *in vivo* to sites of the SF-25 antigen include ^{212}Bi, ^{131}I, ^{125}O, ^{186}Re, and ^{90}Y, which list is not intended to be exhaustive. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy.

Cytotoxic drugs which can be conjugated to the c-SF-25 MAbs of the present invention and subsequently used for *in vivo* therapy include, but are not limited to: alkylating agents such as nitrogen mustards (e.g., cyclophosphamide), ethylenimines (e.g., thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., lomustine and semustine) and triazenes (e.g., dacarbazine); antimetabolites such as folic acid analogs (e.g., methotrexate), pyrimidine analogs (e.g., 5-fluorouracil and fluorodeoxyuridine), and purine analogs and related inhibitors (e.g., 6-mercaptopurine, and pentostatin); natural products such as vinca alkaloids (e.g., vinblastine and vincristine), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, and mitomycin C), enzymes (e.g., L-asparaginase), and biological response modifiers (e.g., interferon alpha); miscellaneous agents

such as platinum coordination complexes (e.g., cisplatin), anthracenediones (e.g., mitoxantrone), substituted ureas (e.g., hydroxyurea), methy hydrazine derivatives (e.g., procarbazine), adrenocortical suppressants (e.g., aminoglutethimide); and hormones and antagonists, such as adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone), estrogens (e.g., ethinyl estradiol), antiestrogens (e.g., tamoxifen), androgens (e.g., fluoxymesterone), antiandrogens (e.g., flutamide) and/or gonadotropin-releasing hormone analogs (e.g., leuprolide). Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and protein synthesis. For a fuller exposition of these classes of drugs which are known in the art, and their mechanisms of action, see Calabresi, P., *et al.*, Chemotherapy of Neoplastic Diseases (pp. 1202-1208) and Antineoplastic Agents (pp. 1209-1263), both in *Goodman and Gilman's THE PHARMACOLOGICAL BASIS OF THERAPEUTICS* (Gilman, A.G., *et al.*, eds.), 8th Edition, Pergamon Press, New York, NY (1990).

One preferred type of toxin molecule is a "photo-activatable toxin molecule." Examples of such "photo-activatable toxin molecules" include Photofrin II (Williams et al., *Photochem. Photobiol. 46*:733-738 (1987); Mattielli et al., *Photochem. Photobiol. 46*:873-880 (1987)), hematoporphyrin derivatives (Benson, R.C., *Urology 31*:13-17 (1988)), hemoglobin, and its derivatives (Polla et al., *Ann. Dermatol. Venereol. 114*:497-505 (1987)); procion blue (Macklis et al., *Brain Res. 359*:158-165 (1985)), fluorescene, and other dyes (Miller et al., *Science 206*:702-704 (1979); Manyak et al., *J. Clin. Oncol. 6*:380-391 (1988)), etc. The critical attribute of such molecules is that they are capable of greater absorption of light (at some wavelength) than the surrounding tissue.

In this therapy, termed "photothermolysis," photo-activation of the toxin is achieved by a careful selection of wavelength, pulse, and intensity of the light. The light energy absorbed by such molecules is released either as heat or emitted as light at a different wavelength. If a suitable light (such as, preferably, a laser light) is employed, the death of cells and tissue which

contain the photo-activatable toxin will occur, either because of the amount of the heat released by this process, or because of the photo-oxidation of essential biological molecules in the cells or tissue by the emitted light. The physics of laser therapy and photothermolysis are reviewed by Hobbs et al. (*J. Dermatolog. Surg. Oncol. 13*:955-964 (1987)), Anderson *et al.* (*Science 220*:524-527 (1983)), Macklis *et al.* (*Brain Res. 359*:158-165 (1985)), Wilson, B.C. (*Phys. Med. Biol. 31*:327-360 (1986)), and especially by Manyak *et al.* (*J. Clin. Oncol. 6*:380-391 (1988)), all of which are incorporated herein by reference.

By conjugating photo-activatable toxins with the chimeric MAbs of the present invention, it is possible to direct the toxin molecule only to those cells which express the SF-25 antigen. Conjugating photo-activatable toxins to antibodies has been used to provide a selective means for treating a tumor without damage to normal (i.e. non-antigen expressing) cells. Examples of the use of this method are provided by: Mew *et al. (Cancer Res. 45*:4380-4386 (1985); *J. Immunol. 130*:1473-1477 (1983)); by Wat *et al., Prog. Clin. Biol. Res. 170*:351-360, (Doiron *et al.* eds.), Alan R. Liss, NY (1984)); and by Oseroff *et al. (Photochem. Photobiol. 46*:83-96 (1987)); *Id. 43 Suppl.*:105s (1986); *Id. 41 Suppl.*:75s (1985); *Id. 41 Suppl.*:35s (1985); *Proc. Natl. Acad. Sci. (USA) 83*:8744-8748 (1986); *Clin Res. 33*:674a (1985); *J. Invest. Dermatolog. 84*:335 (1985)); all of which references are herein incorporated by reference.

The above-described photothermolysis therapy can be accomplished using any light source which is capable of photo-activating the toxin. The photo-activation of such toxins can thus be achieved using light sources other than lasers. For example, such photo-activation can be achieved using light from an ordinary light bulb (Dougherty *et al., J. Natl. Canc. Inst. 55*:115-129 (1979); Wilson, B.C., *Phys. Med. Biol. 31*:327-360 (1986)). Photo-activation of the toxin may alternatively be achieved by the administration of a chemiluminescent agent (i.e. a light-emitting molecule) to an individual who has recieved the photo-activatable toxin. This embodiment of the present

invention is particularly advantageous in the *in situ* treatment of gastric carcinomas, intestinal polyps, and Barrett's esophagus. This embodiment may also be used for metastatic cancers (Phillip *et al.*, In: Porphyrin Localization and Treatment of Tumors (Doiron *et al.*, eds.), Alan R. Liss, NY, pp. 563-569 (1985)).

The therapeutic compositions which contain the c-SF-25 MAbs of the present invention, and/or their conjugates just described, can be administered orally or parenterally by the intravenous, intramuscular, subcutaneous, rectal, transdermal, intrapulmonary, intraperitoneal, intrathecal, intratumoral, intranasalpharyngeal or other known routes of administration. These therapeutic compositions will be manufactured by methods which are well known to those of skill in the art.

As would be understood by one of ordinary skill in the art, such therapeutic compositions may contain salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the c-SF-25 MAb and its conjugates.

Compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Examples of non-aqueous solvents are propylene glycol, polyethylene gylcol, vegetable oils, such as olive oil, and injectable organic esters such as ethyl oleate.

Carriers or occlusive dressings can be used to increase skin permability and enhance antibody absoprtion.

Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water.

Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents.

Adjuvants are substances that can be used to specifically augment a specific immune response. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the animal being immunized. Adjuvants can be loosely divided into several groups based upon their composition. These groups include oil adjuvants (for example, Freund's complete and incomplete), mineral salts (for example, $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, silica, kaolin, and carbon), polynucleotides (for example, poly IC and poly AU acids), and certain natural substances (for example, wax D from *Mycobacterium tuberculosis*, as well as substances found in *Corynebacterium parvum*, or *Bordetella pertussis*, and members of the genus *Brucella*). Among those substances particularly useful as adjuvants are the saponins such as, for example, Quil A. (Superfos A/S, Denmark). Examples of materials suitable for use in vaccine compositions are provided in *Remington's Pharmaceutical Sciences* (Osol, A., Ed., Mack Publishing Co., Easton, PA, pp. 1324-1341 (1980)).

Treatment of an individual with a tumor bearing the SF-25 antigen recognized by the c-SF-25 MAbs of this invention comprises admininstering an effective amount of this c-SF-25 MAb, or fragments or derivatives thereof in a single dose, multiple doses or an infusion of these chimeric MAbs and/or their conjugates to a patient or other animal.

According to the present invention, an "effective amount" of a therapeutic composition is one which is sufficient to achieve the desired biological effect. Generally, the dosage needed to provide an effective amount of the composition, and which can be adjusted by one of ordinary skill in the art, will vary depending upon such factors as the individual chimeric antibody used, the presence and nature of any therapeutic agent conjugated thereto, the

animal's or patients age, condition, sex, and clinical status including extent of disease, if any, and other variables.

The effective dosage can vary from about 10 ng/kg body weight to about 100 mg/kg body weight. Effective concentrations of the compositions of the invention can vary from 0.01-1,000 μg/ml per dose.

E. The SCID Mouse Model

Since hepatic metastases are often a complication of colorectal cancer, a SCID mouse model was established using LS 180 human colon adenocarcinoma cells to evaluate the potential anti-tumor effects of chimeric SF-25. In an attempt to provide a control for the human IgG1 Fc component of this chimeric MAb in functional studies, the results were compared to other chimeric MAbs.

The SCID mouse model is useful because: 1) the blood supply to the tumor cells in the liver is substantially better than that to tumor cells grown in previously described models that used subcutaneous tumor xenografts; and 2) the SCID mouse lacks both T and B cells and therefore will accept xenografts of normal and tumor human tissues (*Proc. Curr. Top. Microbiol. Immunol. 152*:1-263 (1989); Bosma *et al., Ann. Rev. Immunol. 9*:323-350 (1991); McCune *et al., Science 241*:1632-1639 (1988); Mosier *et al., Nature 335*:256-259 (1988)).

Applicants have produced a mouse-human c-SF-25 MAb and demonstrated that: the c-SF-25 MAb selectively binds to the SF-25 antigen; the c-SF-25 MAb induced ADCC by human PBLs of LS 180 human-derived tumor cells; the c-SF-25 MAb inhibited the development of hepatic metastases of human colon adenocarcinoma cells; mice treated with c-SF-25 MAb had a statistically significant increased survival rate compared to control mice injected with LS 180 cells; and that the stable binding of c-SF-25 MAb to the tumor cell surface by its antigen binding site at physiological temperature (37°C) is important for its anti-tumor effect.

Having now generally described the invention, the same will be more readily understood through reference to the following methods and examples, which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1

Preparation of Chimeric SF-25 MAb

Cell Lines

The murine hybridoma cell line SF-25 secretes a monoclonal antibody of IgG1 heavy chain and Kappa light chain isotype that binds to a 125 kD cell surface antigen called the SF-25 antigen which is found on a variety of human adenocarcinoma cell lines of endodermal origin. It was obtained by fusing SP2/0-AG14 cells with spleen cells from a Balb/c mouse which had been immunized with the human hepatocellular carcinoma cell line FOCUS.

SP2/0-AG14 (SP2/0), and SF-25 cells were grown in Iscove's modified Dulbecco's medium (Hazleton Biologics, Inc., Lenexa, KS) supplemented with 5% FCS (Cell Culture Laboratories, Cleveland, OH) and 2 mM L-glutamine (Hazleton).

Purification of the Chimeric SF-25 Antibody

Cell culture supernatant was adjusted to 2 mM EDTA, 140 mM NaCl, and 20 mM Tris pH 8.5 and applied to a protein A sepharose column (Pharmacia, Piscataway, NJ). Chimeric SF-25 IgG1 antibodies was eluted with 0.1 M sodium citrate pH 3.5, neutralized with 1M Tris base and dialyzed against PBS.

Cloning of the SF-25 Variable Region Genes

High molecular weight DNA and cytoplasmic RNA was isolated from SF-25 cells by standard procedures. For Southern blot analysis, genomic DNA was digested with restriction endonucleases (New England Biolabs, Beverly, MA), fractionated by electrophoresis through a 0.8% agarose gel, and transferred to MagnaGraph (MSI, Westborough, MA). dCTP ^{32}P-labeled probes were prepared using a random priming labeling kit (Boehringer Mannheim, Indianapolis, IN).

To clone the heavy chain variable region, 150 μg of SF-25 DNA was digested with EcoR1 and size fractionated on a preparative 0.8% agarose gel. DNA fragments 4-5 kb in size were excised, electroeluted, ligated to EcoR1 digested lambda gt10 (Promega Biotech, Madison, WI) and packaged *in vitro* (Stratagene, LaJolla, CA). Twenty thousand plaques per 150 MM plate were screened using a J_H probe specific for the J segment of the murine heavy chain locus. To clone the light chain variable region, 150 μg of SF-25 DNA was digested with HindIII and size-fractionated on a preparative 0.8% agarose gel. DNA fragments 3-4 kb in size were excised, electroeluted, ligated to HindIII-digested Charon 27 and packaged *in vitro*. The phages were screened as above using a J_K probe specific for the murine J region of the kappa locus. Hybridizations were in 5X SSC (1X SSC = 0.15 M NaCl/0.015 M sodium citrate), 2X Denhardt's (1X Denhardt's = 0.02% each Ficoll 400, polyvinyl pyrrolidine and bovine serum albumin), 0.1% SDS, 200 μg/ml salmon sperm DNA, and 50% (vol/vol) formamide. Final washes were in 0.5X SSC at 65°C. Positive clones were plaque purified by three rounds of screening.

Generation of Chimeric Antibody-Producing Cell Lines

Vector pSF25kapgpt (Figure 2A) and pSF25 G1 apgpt (Figure 2B) were linearized with BamHI and transfected into the non-producing mouse myeloma cell line SP2/0 by electroporation using a BioRad Gene Pulser (Bio-

Rad Laboratories, Richmond, CA). $2x10^7$ cells were suspended in 0.8 ml Hanks buffered saline solution with 10 μg of each expression plasmid, previously digested with BamHI, and subjected to a pulse of 200 volts.

Cells were placed on ice for 10 minutes and plated at a density of 1 x 10^5 cells/ml in 96 well microtiter dishes. Selection was applied 48 hours later (0.5 μgml mycophenolic acid, 2.5 μg/ml hypoxanthine, 50 μg/ml xanthine; Sigma Chemical Company, St. Louis, MO) and resistant colonies were expanded into stable cell lines.

Clones were assayed for antibody production by an Elisa assay using goat-anti-human IgG (Fc Fragment specific) coating antibody and goat anti-human IgG (H+L) alkaline phosphatase conjugated antibody (Jackson ImmunoResearch, West Grove, PA). Standard curves were generated using chimeric antibodies purified by protein A Sepharose chromatography. The highest producing cell lines were subcloned by limiting dilution and evaluated for antibody production in T75 flasks. Growth curves were obtained by seeding cultures at 1 x 10^5 cells/ml in triplicate T75 flasks and taking daily samples for cell counts and ELISA assays. Chimeric MAb producing cell lines were grown under mycophenolic acid selection (see above) in Iscove's media plus 5% FCS.

Cloning of the SF-25 Light and Heavy Chain Genes

Southern blot analysis of SF-25 DNA was first performed using light and heavy chain specific J region probes. These DNA probes (Figure 1) hybridize to antibody variable region genes that have rearranged to the J locus as well as germline (unrearranged) DNA segments. We compared the hybridization patterns of SF-25 DNA with that seen in DNA from the fusion partner SP2/0. A unique 3.2 kb HindIII fragment in SF-25 hybridized to the light chain probe while a unique 4.5 kb Eco R1 fragment in SF-25 hybridized to the heavy chain probe. These DNA fragments were not in the fusion

partner SP2/0 and most likely represented the functionally rearranged variable region genes.

HindIII or EcoR1 DNA fragments containing the above SF-25 hybridizing fragments were purified from preparative agarose gels, ligated to either HindIII digested Charon 27 (light chain library) or Eco R1-digested λgt10 (heavy chain library), and packaged *in vitro*. Phage clones hybridizing to the immunoglobulin light and heavy chain probes were plaque purified and characterized by restriction enzyme analysis.

A 3.2 HindIII fragment hybridizing to the light chain probe was isolated from the Charon 27 library. Restriction maps of the mouse light chain K region, the light chain probe and the SF-25 light chain 3.2 HindIII fragment are shown in Figure 1A. This 3.2 HindIII fragment was subcloned into the human Kappa light chain expression vector pHuKapgpt, which confers resistance to mycophenolic acid when transfected into mammalian cells.

A 4.5 kb EcoR1 fragment hybridizing to the heavy chain probe was isolated from the λgt10 library. Restriction maps of the mouse heavy chain J region, the mouse heavy chain probe used for hybridization and the SF-25 heavy chain 4.5 Kb EcoR1 DNA fragment are shown in Figure 1B. This 4.5 Kb EcoR1 fragment was subcloned into the human IgG1 heavy chain vector. Both light and heavy chain fragments hybridized to the appropriately sized RNA from cell line SF-25 indicating that they were expressed. The above method of cloning genomic DNA fragments utilizes the endogenous promoters of the SF-25 light and heavy chain genes and maintains the original exon and intron arrangements of each gene.

Expression of Chimeric SF-25 MAb in SP2/0 Cells.

The SF-25 light and heavy chain expression vectors were linearized at the BamHI site and electroporated into the mouse myeloma cell line SP2/0. Transfectants producing antibody were expanded from 96 well plates and were

evaluated for antibody production and stability in T75 flasks over several passages.

Chimeric GA733 (Ross *et al.*, *Biochem. Biophys. Res. Commun.* *135*:297-303 (1986)), 323/A3 (Edwards *et al.*, *Cancer Res.* *46*:1306-1317 (1986)) were prepared.

EXAMPLE 2

Cell Lines

The FOCUS cell line was developoed in Dr. Wand's laboratory (Lun *et al.*, *In Vitro (Rockville)* *20*:493-504 (1984)). All other cell lines were obtained from the American Type Culture Collection (Rockville, MD). All cell lines were maintained in Eagle's MEM (M.A. Bioproducts, Walkersville, MD) supplemented with 10% fetal calf serum inactivated at 56°C for 30 minutes, 10 μM nonessential amino acids, 100 U/ml penicillin, and 100 μg/ml streptomycin. In some experiments cells were maintained in RPMI 1640 (Hazleton Biologics, Inc., St. Lenexa, KS) supplemented with 10% heat-inactivated fetal calf serum, 10 μM nonessential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Cells used for *in vitro* testing were harvested from the monolayer cultures by treatment with 0.04% EDTA/versine buffer in the absence of trypsin for 5 minutes at 37°C. For production of xenografts in nude mice, a human colonic carcinoma cell line, LS 180, was harvested from tissue culture by overlaying subconfluent monolayer cultures with 0.05% trypsin and 0.02% EDTA solution in HBSS (GIBCO Laboratories, Grand Island, NY) for 5 minutes and injected as described below. All the cell lines used were demonstrated to be free of mycoplasma contamination by a nucleic acid hybridization technique using Mycoplasma T.C. 11 (Gen-Probe Inc., San Diego, CA).

Purification and Radiolabeling of Antibodies

The monoclonal antibodies were purified by Sepharose CL-4B Staphylococcal Protein A-affinity column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) and labeled with ^{125}I using the Iodogen method (Fraker *et al.*, *Biochem. Biophys. Res. Commun. 80*:849-857 (1978)). Specific activities of iodinated MAbs were calculated as a ratio of cpm of radionuclide attached per mole of antibody (cpm/mol).

Association Constants

A constant number of LS 180 cells (10^5/well) was incubated with 100 µl of ^{125}I-labeled SF-25 MAb serially diluted with 20% bovine serum/PBS for 4 hours at 4°C or 37°C using 96-well filter-bottomed plates (V & P Scientific Inc., San Diego, CA; Takahashi *et al.*, *Cancer Res. 48*:6573-6579 (1988)). Cells were then washed three times with PBS to remove unbound antibody and bound radioactivity was counted in the gamma well counter. The concentration of MAbs and the amount of bound MAbs were calculated from radioactivity (cpm) and specific activity (cpm/mol) of ^{125}I-labeled MAbs. The association constant (K_A) of MAb and the number of antibody binding sites per tumor cell (Bmax) were determined by the methods and equation described by Frankel and Gerhard (*Molec. Immunol. 16*:191-106 (1979)).

Animals

Four to five week old male athymic Balb/c nude mice were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana). Throughout the experiments, these animals were maintained under specific-pathogen-free-conditions.

Isolation of Human Macrophage and NK-Cells

Venous blood was drawn from healthy volunteers with heparin (200 U/ml in final concentration) and overlain on Ficoll-Paque (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). Mononuclear cells were isolated by centrifuging at 1,200 r.p.m. for 30 minutes at 25°C and were washed three times with RPMI 1640 medium.

Mononuclear cells, adjusted to 5 x 10^6/ml, were incubated on a plastic plate for two hours at 37°C in a CO_2 incubator and macrophages were collected from adherent cells. The purity of the macrophage population was examined by flow cytometric analysis using anti-human monocyte/macrophage MAb, anti-Leu-M3 labeled with FITC (Dimitriu-Bona *et al., J. Immunol. 130*:145-152 (1983); Becton Dickinson Immunocytometry Sys., Mountain View, CA) as described below.

Human NK-cells were isolated by discontinuous density gradient centrifugation as large granular lymphocytes (LGLs) (Timonen *et al., J. Immunol. Method 36*:285-291 (1980)). Non-adherent cells, adjusted to 1 X 10^8 cells/gradient, were separated into fractions by centrifugation at 300 X g for 1 hour at 20°C on a six-step discontinuous density gradient of Percoll (Pharmacia LKB Biotech., Inc., Piscataway, NJ) at concentrations of 37.5, 40.0, 42.5, 45.0, 47.5, and 50% (osmolarity adjusted to 290 mOsm/kg by 10 X PBS). LGLs were collected from the 40.0% concentra-tion of Percoll. The purity of human NK-cells was examined by flow cytometric analysis using FITC labeled anti-Leu-11a, anti-human NK-cell antigen associated with IgG Fc receptor III (FcγRIII, CD16) (Phillips *et al., J. Exp. Med. 159*:993-1008 (1984)) as described below.

Isolation of Murine Peripheral Blood Lymphocytes

Blood was collected from the axillary artery and vein and was transferred to 15 ml Falcon 2099 conical plastic tubes filled with 10 ml of ACT solution (a mixture of 9 volumes of 0.83% NH_4Cl and 1 volume of 0.17M Tris HCl, pH 7.67) containing 200 U of heparin to hemolyze red blood cells. These peripheral blood leukocytes were washed with PBS and were resuspended in 30% metrizamide (Sigma Chemical Co., St. Louis, MO) in HBSS at 4°C at a final ratio of 7 parts metrizamide to 5 parts of packed blood cells in order to isolate lymphocytes. The mixture was transferred into 15 ml conical tubes, overlain with PBS and centrifuged at 1,400 X g for 20 minutes at 4°C. The lymphocyte layer was then carefully removed from the metrizamide-PBS interface and washed three times with PBS.

Preparation of Peritoneal Exudate Cells and Thioglycolate-Elicited Macrophages

Mice were injected intraperitoneally with 5 ml of 0.01 M PBS, pH 7.2. A few minutes later peritoneal exudate cells were obtained by aspirating PBS from the peritoneum cavity. The purity of macrophages was tested by flow cytometry using F4/80 anti-murine macrophage MAb as described below (Austyn *et al., Eur. J. Immunol. 11*:805-815 (1981)).

In order to obtain thioglycolate-elicited macrophages, nude mice were inoculated intraperitoneally with 1 ml of thioglycolate medium (Difco; VWR Scientific Corp., Philadelphia, PA). Four days later, peritoneal exudate cells were collected and were used as thioglycolate-elicited macrophages.

Preparation of Mouse Splenocytes

Mice were anesthetized with ether and were sacrificed by axillary bleeding. The peritoneal cavity was exposed under sterile conditions and the spleen was excised into a plastic dish containing 5 ml of RPMI 1640 medium.

Next, a small incision was made in the spleen and splenocytes were isolated by squeezing repeatedly with forceps. Splenocytes were then transferred into a plastic tube and were washed twice with RPMI 1640 medium.

Isolation of Murine NK-Cells

Murine NK-cells were isolated by discontinuous density gradient centrifugation as a LGL fraction (Lafreniere *et al., Cancer Res. 50*:1658-1666 (1990)). Non-adherent cells were obtained from murine splenocytes after incubation in a plastic dish for 2 hours at 37°C and were adjusted to 5 x 10^7 cells/gradient. They were separated into fractions by centrifugation at 300 X g for 1 hour at 20°C on a seven-step discontinuous density gradient of Percoll at concentrations of 20, 40, 50, 60, 70, and 100%. (The osmolarity was adjusted to 320 mOsm/kg by 10 X PBS). LGLs were collected from the interfaces of 20/40% and 40/50% concentrations of Percoll. The purity of murine NK-cells were examined by flow cytometric analysis using rabbit antiserum to ganglio-N-tetraosylceramide (anti-asialo GM1; Wako Chemicals, Dallas, TX; Kasai *et al., Eur. J. Immunol. 10*:175-180 (1980)) as described below.

Isolation of Lymphocytes from Mouse Liver

Lymphocytes were isolated from murine liver by using the method of Wiltrout *et al. (J. Exp. Med. 160*:1431-1449 (1984)), with some modifications. Mice were anesthetized with ether and sacrificed by axillary bleeding. The peritoneal cavity was exposed under sterile conditions and the portal vein was cannulated with a 27-gauge needle. Five ml of RPMI 1640 containing 10% FCS and antibiotics were injected to flush blood out of the liver through the portal vein. The liver was then excised and the gall bladder was removed. Next, the liver was minced into small pieces with a razor blade and incubated with 10 ml of prewarmed Mg++ free HBSS containing 5%

FCS, Pc 100 IU/ml, SM 100 μg/ml, 5 mM $CaCl_2$, 0.05% collagenase type IV, and 0.002% DNAse I type II (Sigma Chemical Co. St. Louis, MO) for 30 minutes at 37°C with shaking. The liver digest was then filtered through a 50-gauge stainless steel mesh using a sterile syringe plunger, and was washed twice with HBSS. The packed liver digest was then resuspended in 30% metrizamide in HBSS at 4°C at a final ratio of 7 parts metrizamide to 5 parts of packed liver digest. Three ml of the mixture were transferred into 15-ml conical tubes, overlain with 1.5 ml of PBS and centrifuged at 1,400 X g for 30 minutes at 4°C. The lymphocyte layer was then carefully removed from the metrizamide-PBS interface and washed three times with PBS.

Detection of Human Macrophages and NK-Cells by Flow Cytometry

Fifty μl of cell suspension adjusted to 2 x 10^7 viable cells/ml in 0.01 M PBS, pH 7.2 containing 1% BSA and 20 μl of FITC labeled anti-Leu-M3 (anti-human monocyte/macrophage MAb) or anti-Leu-11a (anti-human NK MAb) were incubated for 1 hour at 4°C. After washing three times with PBS, fluorescence was detected in a FACScan (Beckton Dickinson, Mountain View, CA.) for single color flow cytometric analysis. Results are expressed as mean percent of positive staining cells and mean linear fluorescence.

Flow Cytometric Analysis

Murine cells (1 x 10^6) were incubated with 200 μl of rat anti-mouse macrophage MAb F4/80 producing hybridoma culture supernatant (Austyn *et al., Eur. J. Immunol. 11*:805-815 (1981)) for 1 hour at 4°C in a Falcon 2054 plastic tube. After washing three times with cold PBS, the cells were incubated at 4°C for 1 hour with 200 μl of biotin-conjugated anti-rat IgG antibody made in rabbits (Vector Laboratory, Burlingame, CA) adjusted to 7.5 μg/ml in PBS containing 1% BSA. The cells were washed three times with cold PBS, were incubated for another 1 hour at 4°C with 200 μl of avidin

FITC (Becton Dickinson, Mountain View, CA), and were diluted 1:25 with PBS containing 1% BSA. After washing three times with PBS, fluorescence was detected in a Becton Dickinson FACScan. In some experiments macrophages were also examined by non-specific esterase staining.

Alternatively, 1 X 10^6 LS 180 cells were incubated for 1 hour at 4°C with 200 μl of murine SF-25 MAb adjusted to 1 mg/ml in 0.01 M PBS, pH 7.2, containing 1% bovine serum albumin. After washing three times at 4°C, the cells were further incubated at 4°C or 37°C for 15 minutes to 2 hours, and then were reacted for 1 hour at 4°C with fluorescein-conjugated goat anti-mouse IgG (Cappel Lab., Cochranville, PA), diluted 1:100 with 1% bovine serum albumin in PBS. Fluorescence was detected in a Becton Dickinson FACScan for single color flow cytometric analysis. Results are expressed as mean percent of positive staining cells.

Detection of Murine NK Cells by Flow Cytometry

Anti-asialo GM1 rabbit serum was used to detect the NK cells in nude mice by flow cytometry. The cells were collected and adjusted to 5 x 10^6 viable cells/ml in PBS containing 1% BSA. Two hundred μl of this cell suspension and 20 μl of anti-asialo GM1, adjusted to 5 μg/ml, were incubated for 1 hour at 4°C. The cells were washed three times with cold PBS, incubated with 200 μl of FITC-labeled $F(ab')_2$ fragments of goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO), diluted 1:40 with PBS containing 1% BSA were incubated for another one hour at 4°C. Fluorescein bound MAb was detected in a Becton Dickinson FACScan for single color flow cytometric analysis.

ADCC Assay

LS 180 cells were used as the target cells. Confluent cells were harvested with EDTA/Versene buffer and radiolabeled by incubating 1 x 10^6 cells with 100 μCi of sodium chromate-^{51}Cr (New England Nuclear, Boston, MA) for 30 minutes at 37°C. After washing, the radiolabeled cells were adjusted to 1 x 10^5/ml of RPMI 1640. One hundred μl of target cells and 50 μl of MAb (10 μg/ml in RPMI 1640) were pipetted into 96-well U bottom plates and then 100 μl of various concentrations of human PBL (1 x 10^6 - 1 x 10^7 lymphocytes/ml) were added to each well as effector cells. The final concentration of MAb in ADCC was adjusted to 20 μg/ml, since this concentration had been shown to be optimal in preliminary experiments. Plates were incubated in a CO_2 incubator at 37°C for 4 hours when human cells were used as effector cells, and for 8 hours when murine effector cells were used. After centrifugation of the U bottom plates at 1,500 rpm for 15 minutes, 200 μl of culture supernatant was collected and radioactivity was determined by a gamma well counter.

The spontaneous release of ^{51}Cr was measured after the incubation of target cells alone with RPMI 1640 and the total count was determined after the incubation of the cells in 1.0 N HCl. The spontaneous release was less than 10% of the total release in all experiments. The percent specific lysis was determined by the following formula:

$$\frac{\text{Observed cpm - spontaneous cpm}}{\text{Total cpm-spontaneous cpm.}} \times 100 = \% \text{ specific lysis.}$$

Animal Model for Liver Metastases of Human Colon Adenocarcinoma

Nude mice were anesthetized with 0.4 ml of 2% chloral hydrate (Sigma Chemical Co., St. Louis, MO) by ip. injection and placed in the decubitus position. A transverse incision was made in the left flank through the skin and peritoneum, exposing the spleen. Mice were injected with 1.0×10^6 LS 180 cells and 20 μg of rabbit anti-serum to ganglio-N-tetraosylceramide (anti-asialo GM1; Wako Chemicals, Dallas, TX) in 0.25 ml PBS into the portal vein via the splenic hilus using a 27-gauge needle. After waiting for one minute, the portal vein was ligated, the spleen was removed and the abdominal cavity was closed.

In vivo Effect of c-SF-25 MAb

The *in vivo* anti-tumor effect of c-SF-25 MAb was studied using the above described hepatic metastic model. Five days after the injection of LS 180 cells and anti-asialo GM1 antibody, mice were intravenously (iv.) injected with a single dose of c-SF-25 MAb (100 μg/mouse). As a control, an equal molar amount (67 μg/mouse) of $F(ab')_2$ fragment of c-SF-25 MAb was injected into other mice. Thirty days later (five weeks after the tumor cell injection), the mice were sacrificed and examined for metastatic spread in the liver and the presence of local abdominal tumors. Results were statistically significant when p was < 0.05 by the chi-square test with the Yates correction.

In vivo Depletion of Murine Macrophage by Carrageenan

Since iota-carrageenan (iota-CGN; Sigma Chemical Co., St. Louis, MO) is toxic to macrophages (Ishizaka *et al., J. Immunol. 125*:2232-2235 (1980), this reagent was chosen as a macrophage depleting agent. However, native iota-CGN is not water-soluble at low temperatures and the sulfate groups contained in the iota-CGN are mitogenic (Id.) In order to prepare a

water soluble reagent and also to avoid non-specific stimulation of the immune system *in vivo*, iota-CGN was desulfated (DS-CGN) (Ishizaka *et al.*, *J. Immunol. Method 124*:17-24 (1989)) and injected into the nude mice. For *in vivo* depletion of macrophages, 3 mg of DS-CGN dissolved in PBS was injected ip. every other day for 5 days (total of 9 mg/mouse). Preliminary experiments using flow cytometry, demonstrated that peritoneal murine macrophages were almost completely depleted by these injections and that peritoneal exudate cells positive for F4/80 decreased from 89.3% to 9.0%. Macrophages detected in murine peripheral blood were also depleted by the ip injection of DS-CGN and F4/80-positive cells decreased therein from 18.0% to 1.9%. These results show that DS-CGN was effective for the *in vivo* depletion of macrophages.

Survival Rate of Mice with Human Colon Cancer Adenocarcinoma Metastases

Mice with human colon adenocarcinoma metastases developed large hepatic tumors and all animals died within 6 to 7 weeks after the injection of tumor cells due to hepatic dysfunction. Mice were injected iv with c-SF-25 MAb (100 μg/mouse once a week for 4 weeks) starting 5 days after the intraportal injection of tumor cells. The survival rate was compared to the untreated control group using the algorithm of Lee and Desu (*Comput. Prog. Biomed. 2*:315-321 (1972)).

Properties of c-SF-25 MAb

The c-SF-25 showed identical physical properties to the murine MAb with respect to its immunoreactivity and association constant. The binding of ^{125}I-labeled c-SF-25 to the LS 180 cell line was completely inhibited by both cold murine SF-25 and cold c-SF-25 but not by B_2TT, a non-specific MAb (Figure 3). Thus, the chimeric and murine SF-25 MAb recognize the same epitope. Furthermore, the association constants of the chimeric and murine SF-25 MAbs as calculated by Scatchard analysis were 2.21 x 10^8/M and 1.36

x 10^8/M, respectively. These binding constants indicate that the antigen-antibody interaction is of high avidity and that the human/murine chimeric construct of the SF-25 MAb was identical to the murine antibody. Finally, the numbers of antibody binding sites per LS 180 cell were calculated to be approximately 2.5 x 10^5 for both the chimeric and original murine MAb.

ADCC Exhibited by Human Macrophages and NK-cells

ADCC produced by c-SF-25 MAb was studied using human PBL as effector cells (Figure 4A). ADCC was induced at various effector to target cell (E:T) ratios by c-SF-25 but not by murine SF-25 MAb. The specific lysis of colon adenocarcinoma cells by isolated subpopulations of cells was then examined. The purity of macrophages and NK-cells isolated from human PBL were >95% and >90%, respectively, when examined by flow cytometry. Both cell populations induced substantial ADCC against LS 180 cells in the presence of c-SF-25 MAb (Figure 4B) that was greater than that induced by the mixed cellular population of PBLs (Figure 4A).

Animal Model of Hepatic Metastic Disease

Hepatic metastases of human colon adenocarcinoma cells were established by injecting LS 180 cells into the portal vein of nude mice. The administration of anti-asialo GM1 is essential to establish hepatic metastases in nude mice (Takahashi *et al.*, *Gastroenterology* 96:1317-1329 (1989)). However, when anti-asialo GM1 was injected iv. one or two days before tumor cell injection, hepatic tumors developed in only 60% and 40% of the mice, respectively. Therefore, all mice were simultaneously injected iv. with LS 180 cells and anti-asialo GM1 into the portal vein. All these mice developed macroscopic tumors in their livers as well as local abdominal tumors at the site of tumor cell injection.

Effect of Chimeric SF-25 MAb on Tumor Growth *in vivo*

All control mice developed extensive hepatic metastases 5 weeks after tumor cell injection (Group A in Table I). Large "cannon ball-like" multiple tumors developed in the livers of untreated mice (Figure 5A) and 74% of these untreated animals (14 of 19) developed multiple, isolated tumors larger than 5 mm in diameter (Table I).

A single iv. injection of c -SF-25 MAb produced a striking anti-tumor effect. The incidence of mice with hepatic metastases was significantly reduced from 100% (19 of 19) to 22% (2 of 9) ($P<0.01$: c-SF-25 treated vs controls; Table I) and most of these mice were free of detectable hepatic tumors (Figure 5B). Two mice treated with c-SF-25 MAb developed hepatic tumors, but their tumor burden was substantially less than the controls and all tumors were less than 3mm. c-SF-25 MAb also inhibited local abdominal tumor growth ($P<0.005$ compared to controls).

In contrast to the striking anti-tumor effect exhibited by c-SF-25 MAb, the $F(ab')_2$ fragment had little effect on colon adenocarcinoma cell growth. The mice treated with $F(ab')_2$ fragment developed multiple hepatic tumors larger than 5 mm in diameter (Group C in Table I) and were similar to control

mice (Figure 5C). The F(ab')$_2$ fragment had little effect on the local abdominal tumors (Table I).

Table I. *In Vivo* Anti-Tumor Effect of c-SF-25 MAb on Human Colon Adenocarcinoma Growth In Nude Mice

Group	A		B		C	
Treatment	None (n=19)		c-SF-25 MAb (n = 9)		F(ab')$_2$ of c-SF-25 (n = 10)	
Hepatic metastasis	19/19	(100%)	2/9	(22%)*	8/10	(80%)
<3 mm†	0/19	(0%)	2/2	(100%)	0/8	(0%)
3-5 mm	5/19	(26%)	0/2	(0%)	0/8	(0%)
>5 mm	14/19	(74%)	0/2	(0%)	8/8	(100%)
Local abdominal tumor	19/19	(100%)	4/9	(44%)§	9/10	(90%)
<3 mm	0/19	(0%)	4/4	(100%)	1/9	(11%)
3-5 mm	0/19	(0%)	0/4	(0%)	0/9	(0%)
>5 mm	19/19	(100%)	0/4	(0%)	8/9	(89%)

* $p<0.001$ vs. no treatment.
† Tumor size as measured by largest diameter of an individual tumor.
§ $p<0.005$ vs. no treatment.

In vivo Effect of c-SF-25 MAb after Macrophage Depletion

In order to test if macrophages were involved in the *in vivo* anti-tumor effect produced by c-SF-25 MAb, DS-CG was injected ip. to deplete murine macrophages after the injection of the LS 180 tumor cells. The DS-CGN basically did not affect the development of hepatic metastases (Group A v. Group B in Table II). The anti-tumor effect of c-SF-25 MAb (100 μg/mouse) on hepatic metastases was reduced by DS-CGN and hepatic metastases were present in all mice treated with c-SF-25 MAb (Group C, Table II). However, hepatic metastases of mice treated with c-SF-25 MAb and DS-CGN were still significantly smaller than those of untreated animals ($P<0.01$; Group C v. Group A in Table II). In contrast, the anti-tumor effect of c-SF-25 MAb on local abdominal tumors was completely blocked by DS-CGN and mice developed extrahepatic tumors similar in size to untreated mice (Group C v. Group A in Table II).

Analysis of Liver Infiltrating Lymphocytes

Three weeks after their LS 180 tumor cell injections, mice were injected iv. with c-SF-25 MAb (100 μg/mouse). The mice were sacrificed the following day. Flow cytometric analysis of liver infiltrating cells detected by anti-asialo-GM1 rabbit serum demonstrated a larger population of NK-cells in the liver (31%) than in peripheral blood (7%) in mice with liver tumors and also in normal animals (36% vs. 5%, respectively; Table III)). There was no significant difference in the macrophage population size, as determined by non-specific esterase activity between liver (25%) and peripheral blood (15%; Table III).

Table II. **Anti-tumor effect of c-SF-25 MAb after *in vivo* depletion of murine macrophages by desulfated iota-carrageenan**

Group	A		B		C	
DS-CGN*	-		+		+	
c-SF-25 MAb	-		-		+	
	(n = 13)		(n = 10)		(n = 10)	
Hepatic metastases	13/13	(100%)	10/10	(100%)	10/10	(100%)
<3 mm	0/13	(0%)	2/10	(20%)	5/10	(50%)
3-5 mm	5/13	(38%)	0/10	(0%)	4/10	(40%)
>5 mm	8/13	(62%)	8/10	(80%)	1/10	(10%)†
Local abdominal tumor	13/13	(100%)	8/10	(80%)	9/10	(90%)
<3 mm	0/13	(0%)	0/8	(0%)	0/9	(0%)
3-5 mm	0/13	(0%)	0/8	(0%)	0/9	(0)%)
>5 mm	13/13	(100%)	8/8	(100%)	9/9	(100%)

* Desulfated iota-carrageenan (DS-CGN).

† $p < 0.01$ vs. no treatment.

Table III. Analysis of Liver Infiltrating Cells

Hepatic metastasis		+	-
Liver infiltrating cells	Anti-asialo GM1 positive	31.0%	36.0%
	Non-specific esterase positive	25.0%	20.0%
Peripheral blood cells	Anti-asialo GM1 positive	7.0%	5.0%
	Non-specific esterase positive	15.0%	18.0%

ADCC Produced by Murine Macrophages and NK-Cells

ADCC was induced by c-SF-25 MAb using thioglycolate-elicited murine macrophages as the effector cells at various E:T ratios (Figure 6A). Cytotoxicity was also produced when [^{3}H]methyl-thymidine labeled LS 180 cells were used as target cells in a longer term cytotoxicity assay (12-24h; data not shown). ADCC was induced by murine splenocytes in the presence of c-SF-25 MAb (Figure 6B).

NK-cells were isolated from these murine splenocytes by discontinuous density gradient centrifugation. The purity of these NK-cells, as determined by flow cytometry, was over 90%. These purified murine NK-cells demonstrated ADCC in the presence of c-SF-25 MAb. (Figure 6C).

Effect of c-SF-25 MAb on Animal Survival

All control mice died within 6 weeks after tumor cell injection due to massive hepatic metastases and liver dysfunction (Figure 7). In contrast, all mice administered c-SF-25 MAb survived more than 6 weeks and 60% of them survived more than 9 weeks. Median survival of the control and treated groups were 37 $\pm$ 4 days and 79 $\pm$ 35 days, respectively. The improved survival rate of the c-SF-25 MAb treated animals was highly significant ($P < 0.0002$).

Properties of Chimeric Antibodies

Binding studies were performed. The c-SF-25 MAb association constant was $2.2 \times 10^8\ M^{-1}$. The number of SF-25 and binding sites per cell were 2.3×10^5/cell when studied at 4°C using LS 180 cells as the antigen source.

Functional studies with chimeric SF-25 MAbs were performed in order to determine if the human IgG1 isotype could induce ADCC activity by murine effector cells. ADCC was induced at various effector to target cell (E:T) ratios greater than 12.5:1 only in the presence of chimeric MAbs (20μg/ml; Figure 8). Murine splenocytes alone had no cytotoxicity against LS 180 cells.

In vivo Effect of Chimeric Antibodies

An animal model of hepatic metastases was employed to test the anti-tumor effects of chimeric SF-25 MAb. All control mice injected with LS-180 cells as previously described developed both hepatic and local abdominal tumors (Table 4). The typical tumor growth pattern in a control animal sacrificed 5 weeks after tumor cell injection showed large "cannon-ball" like tumors throughout the liver.

A single intravenous injection of chimeric SF-25 MAb substantially inhibited LS-180 tumor growth. The percent of mice bearing hepatic metastases was reduced from 100% to 22% ($P < 0.001$: vs. controls; Table 4) and most were entirely free of detectable hepatic disease. Two animals in the SF-25 MAb treatment group developed hepatic tumors at 5 weeks, but the size of these tumors was considerably smaller than in the controls (Table 4). Local abdominal tumors were also reduced in the SF-25 treatment group (100% to 44%, $P < 0.025$; Table 4).

Table 4. ***In vivo*** **anti-tumor effect of chimeric SF-25 MAb on human colon adenocarcinoma growth in nude mice.**

Treatment	None (n = 13)		c-SF-25 MAb (n = 9)	
Hepatic metasteses	13/13	(100%)	2/9	(22%)*
< 3 mm†	0/13	(0%)	2/9	(22%)
3-5 mm	5/13	(38%)	0/9	(0%)
> 5 mm	8/13	(62%)	0/9	(0%)
Local abdominal tumor	13/13	(100%)	4/9	(44%)§

* $P < 0.01$ vs. no treatment.
† Largest tumor size as measured by diameter of individual tumors.
§ $P < 0.025$ vs. no treatment.

Chimeric Antibody Binding Studies

Since the *in vitro* and *in vivo* experiments were performed at 4°C and under physiological conditions (37°C), respectively, direct binding assays of ^{125}I-labeled SF-25 MAbs to LS 180 tumor cells were performed at different temperatures. The specific binding of SF-25 to the tumor cell surface was significantly greater at 37°C than at 4°C ($P < 0.001$; Figure 9).

The binding of SF-25 MAb to the tumor cell surface was relatively stable both at 4°C and at 37°C (Table 5).

Table 5. Flow cytometric analysis of monoclonal antibody binding to tumor cells

		Mean percent of positive staining cells (%)			
		0 min	15 min	30 min	120 min
Murine SF-25	4°C	91.4	90.2	90.7	91.8
	37°C	91.4	90.0	82.4	66.4

Direct binding investigations using different MAbs demonstrated that at 37°C, the specific binding of both GA733 and 323/A3 MAbs increased (Figure 10). Because the binding of GA733 and 323/A3 appeared to be stable at 37°C, these data indicate that the antigen recognized by both MAbs (GA733 and 323/A3) was not shed from the cell surface.

To confirm and extend these observations, LS 180 tumor tissue was placed in various fixatives to immobilize the antigen in the membrane. ^{125}I-labeled GA733 and 323/A3 MAbs reacted with LS 180 tumor tissue at 4°C. Furthermore, there was no temperature effect on the binding of GA733 and 323/A3 MAbs to LS 180 derived tumor tissue. Identical results were obtained when LS 180 tumors were fixed with 2% paraformaldehyde or 4% formaldehyde at various times and temperatures.

Association Constants of Monoclonal Antibodies

The binding association constants of chimeric and murine SF-25, GA733 and 323/A3 MAb remained unchanged at 37°C compared to 4°C (Table 6).

ADCC Induced by Various Concentrations of Chimeric Antibodies

The ADCC activities produced by chimeric SF-25 at various antibody concentrations were studied. Chimeric SF-25 MAbs induced ADCC mediated by murine NK-cells purified from a splenocyte derived population at 37°C in the presence of low and high concentrations of antibody (20μg/ml; Figure 11A). Thioglycollate elicited macrophages mediated ADCC at both low and high concentrations of chimeric SF-25 MAbs (Figure 11B).

Table 6. Association constants of monoclonal antibodies

MAb	4°C K_A ($X10^8M^{-1}$)	37°C K_A ($X10^8M^{-1}$)
chimeric SF-25	2.21	4.86
murine SF-25	1.42	1.26
murine GA73-3	0.42	0.65
murine 323A3	0.39	0.68

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 1, line 10

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
08 December 1991	HB 9599

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

Murine Hybridome Cell Line, SF-25

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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WHAT IS CLAIMED IS:

1. A polynucleotide molecule comprising a sequence coding for the variable region of an immunoglobulin chain having specificity to the antigen bound by the murine SF-25 antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599 and which further comprises an additional sequence coding for at least part of the constant region of a human immunoglobulin chain, both said sequences in operable linkage with each other.

2. The molecule of claim 1 wherein said chain is a heavy chain.

3. The molecule of claim 1 wherein said chain is a light chain.

4. The molecule of claim 1 which is a recombinant DNA molecule.

5. The molecule of claim 4 which is in double stranded DNA form.

6. The molecule of claim 5 which is an expression vehicle.

7. The molecule of claim 6 wherein said vehicle is a plasmid.

8. A prokaryotic host transformed with the molecule of claim 1.

9. The host of claim 8 which is a bacterium.

10. A eukaryotic host transfected with the molecule of claim 1.

11. The host of claim 10 which is a yeast cell or a mammalian cell.

12. A chimeric immunoglobulin heavy chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

13. A chimeric immunoglobulin light chain comprising at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599.

14. A chimeric antibody molecule comprising two light chains and two heavy chains, each of said chains comprise at least part of a human constant region and at least part of a variable region having specificity to the antigen bound by the murine SF-25 monoclonal antibody given the ATCC designation HB 9599, or a fragment or derivative of said chimeric antibody.

15. The chimeric immunoglobulin heavy chain of claim 12 wherein said heavy chain is detectably labeled.

16. The chimeric immunoglobulin light chain of claim 13 wherein said light chain is detectably labeled.

17. The antibody, fragment or derivative thereof, of claim 14 wherein said antibody, fragment or derivative thereof, is detectably labelled.

18. The molecule of any of claims 15, 16 or 17 wherein said detectable label is an enzyme label.

19. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a radioisotopic label.

20. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a nuclear magnetic resonance contrasting agent.

21. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a nonradioactive isotopic label.

22. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a fluorescent label.

23. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a toxin label.

24. The molecule of any of claims 15, 16 or 17 wherein said detectable label is a chemiluminescent label.

25. The chimeric immunoglobulin heavy chain of claim 12 wherein said heavy chain is conjugated with a therapeutic molecule.

26. The chimeric immunoglobulin light chain of claim 13 wherein said light chain is conjugated with a therapeutic molecule.

27. The antibody, fragment or derivative thereof, of claim 14 wherein said antibody, fragment or derivative thereof, is conjugated with a therapeutic molecule.

28. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is a toxin molecule.

29. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is radionuclide.

30. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is cytotoxic drug.

31. The molecule of any of claims 25, 26, or 27 wherein said therapeutic molecule is photo-activatable toxin.

32. A process for preparing a chimeric immunoglobulin heavy chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599,comprising:

(a) culturing a host capable of expressing said heavy chain under culturing conditions,

(b) expressing said heavy chain; and

(c) recovering said heavy chain from said culture.

33. A process for preparing a chimeric immunoglobulin light chain having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising:

(a) culturing a host capable of expressing said light chain under culturing conditions;

(b) expressing said light chain; and

(c) recovering said light chain from said culture .

34. A process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the murine SF-25 monoclonal antibody secreted by the hybridoma cell line given the ATCC designation HB 9599 comprising:

a. culturing a host capable of expressing said light chain under culturing conditions, expressing said light chain, and recovering said light chain from said culture;

(b) separately culturing a host capable of expressing said heavy chain under culturing conditions, expressing said heavy chain, and recovering said heavy chain from said culture; and

(c) associating said recovered heavy chain and light chain, thereby preparing said chimeric immunoglobulin, fragment or derivative.

35. A process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the murine hybridoma cell line given the ATCC designation HB 9599, comprising:

(a) co-culturing a host capable of expressing said heavy chain with a host capable of expressing said light chain under culturing conditions;

(b) expressing said heavy chain and said light chain;

(c) permitting said heavy chain and said light chain to associate into said chimeric immunoglobulin, fragment or derivative; and

(d) recovering said chimeric immunoglobulin, fragment or derivative from said culture.

36. A process for preparing a chimeric immunoglobulin, fragment or derivative, containing a heavy chain and a light chain, each of said heavy and light chains having at least part of a human constant region and at least part of a variable region with specificity to the antigen bound by the SF-25 murine monoclonal antibody secreted by the hybridoma cell line given the ATCC designatio HB 9599, comprising:

(a) culturing a host capable of expressing said heavy chain and said light chain under culturing conditions;

(b) expressing said chimeric immunoglobulin, fragment or derivative and

(c) recovering from said culture said chimeric immunoglobulin, fragment or derivative.

37. The process of any of claims 32-36 wherein said host is prokaryotic.

38. The process of any of claims 32-36 wherein said host is eukaryotic.

39. An immunoassay method for detecting an antigen capable of binding to the chimeric SF-25 monoclonal antibody in a sample comprising:

(a) contacting said sample with the detectably labeled antibody, fragment or derivative of any of claims 17-23 or 24; and

(b) detecting said antigen by detecting the binding of said antigen to said antibody, fragment or derivative thereof.

40. An imaging method for detecting a tissue antigen capable of binding to the chimeric SF-25 monoclonal antibody, comprising:

(a) contacting the detectably labeled antibody, fragment or derivative of any of claims 17-23 or 24 with said tissue; and

(b) detecting said antigen.

41. The method of claim 40, wherein said contacting occurs *in vivo* within the body of an animal.

42. The method of claim 41, wherein said animal is a human being.

43. The method of claim 40, wherein said contacting occurs *in vitro* outside the body of an animal.

44. The method of claim 40 wherein said tissue is colon adenocarcinoma tissue.

45. The method of claim 40 wherein said tissue is hepatic metastatic tissue derived from colon adenocarcinoma tissue.

46. The use of an effective dose of a chimeric SF-25 monoconal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for the killing of cells expressing an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody.

47. The use of claim 46, wherein said killing occurs *in vivo* within the body of an animal.

48. The use of claim 47, wherein said animal is a human being.

49. The use of claim 46, wherein said cells are of endodermal origin.

50. The use of claim 49, wherein said cells are colon adenocarcinoma cells.

51. The use of claim 50, wherein said cells are cells of hepatic metastases which are derived from said colon adenocarcinoma cells.

52. The use of claim 46, wherein said killing occurs by antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, radiation cytotoxicity, cytotoxic drug action, or toxin action.

53. The use of claim 46,wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

54. The use of claim 46, wherein said fragment is a single chain antibody binding protein.

55. The use of claim 46, wherein said chimeric SF-25 monoclonal antibody is a humanized SF-25 monoclonal antibody.

56. The use of claim 55, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of variable domains of an antibody which recognizes the SF-25 antigen defined by the murine SF-25 antibody.

57. The use of an effective dose of a chimeric SF-25 monoclonal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for treating an animal having a tumor expressing an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody.

58. The use of claim 57, wherein said animal is a human being.

59. The use of claim 57, wherein said tumor is of endodermal origin.

60. The use of claim 59, wherein said tumor is a colon adenocarcinoma.

61. The use of claim 60, wherein said tumor is a hepatic metastatic tumor which is derived from said colon adenocarcinoma.

62. The use of claim 57, wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

63. The use of claim 57, wherein said fragment is a single chain antibody binding protein.

64. The use of claim 57, wherein said chimeric SF-25 monoclonal antibody is a humanized SF-25 monoclonal antibody.

65. The use of claim 64, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of variable domains of an antibody which recognizes the SF-25 antigen defined by the murine SF-25 antibody.

66. The use of an effective dose of a chimeric SF-25 monoclonal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for treating an animal which has pancreatic carcinoma or other pancreatic cancer, which expresses an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody.

67. The use of claim 66, wherein said antibody is a chimeric SF-25 monoclonal antibody.

68. The use of claim 66, wherein said antibody is a humanized SF-25 monoclonal antibody.

69. The use of claim 68, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of the variable domains of an antibody which recognizes the SF-25 antigen as defined by murine SF-25 antibody.

70. The use of claim 66, wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

71. The use of claim 66, wherein said fragment is a single chain antibody binding protein.

72. The use of claim 66, wherein said treatment occurs *in vivo* within the body of the animal.

73. The use of claim 72, wherein said animal is a human.

74. The use of an effective dose of a chimeric SF-25 monoclonal antibody, or fragment or derivative thereof, of any of claims 14, 25, 26 or 27 for treating an animal having cancer which expresses an antigen, which antigen is capable of binding to said chimeric SF-25 monoclonal antibody, with the cancer selected from the group consisting of colon adenocarcinoma, hepatocellular carcinoma, cholangiocellular carcinoma, gastric adenocarcinoma, rectal adenocarcinoma, breast adenocarcinoma, bladder adenocarcinoma, squamous cell carcinoma of the lungs, adenocarcinoma of the lungs, large cell carcinoma of the lungs, small cell carcinoma of the lungs, lymphoproliferative disease, myeloproliferative disease, lymphoma, leukemia, kidney carcinoma, ovary adenocarcinoma, cervical carcinoma, uterine endometrial adenocarcinoma, liver hepatoma, choriocarcinoma, malignant meloma, including the primary tumors or metastases or micrometastases of these diseases.

75. The use of claim 74, wherein said antibody is a chimeric SF-25 monoclonal antibody.

76. The use of claim 74, wherein said antibody is a humanized SF-25 monoclonal antibody.

77. The use of claim 76, wherein said humanized SF-25 monoclonal antibody is a human immunoglobulin grafted with complementarity-determining regions (CDRs) of the variable domains of an antibody which recognizes the SF-25 antigen as defined by murine SF-25 antibody.

78. The use of claim 74, wherein said fragment is a Fab, Fab', $F(ab')_2$, or Fv fragment.

79. The use of claim 74, wherein said fragment is a single chain antibody protein.

80. The use of claim 74, wherein said treatment occurs *in vivo* within the body of the animal.

81. The use of claim 74, wherein said animal is a human being.

FIG.1A

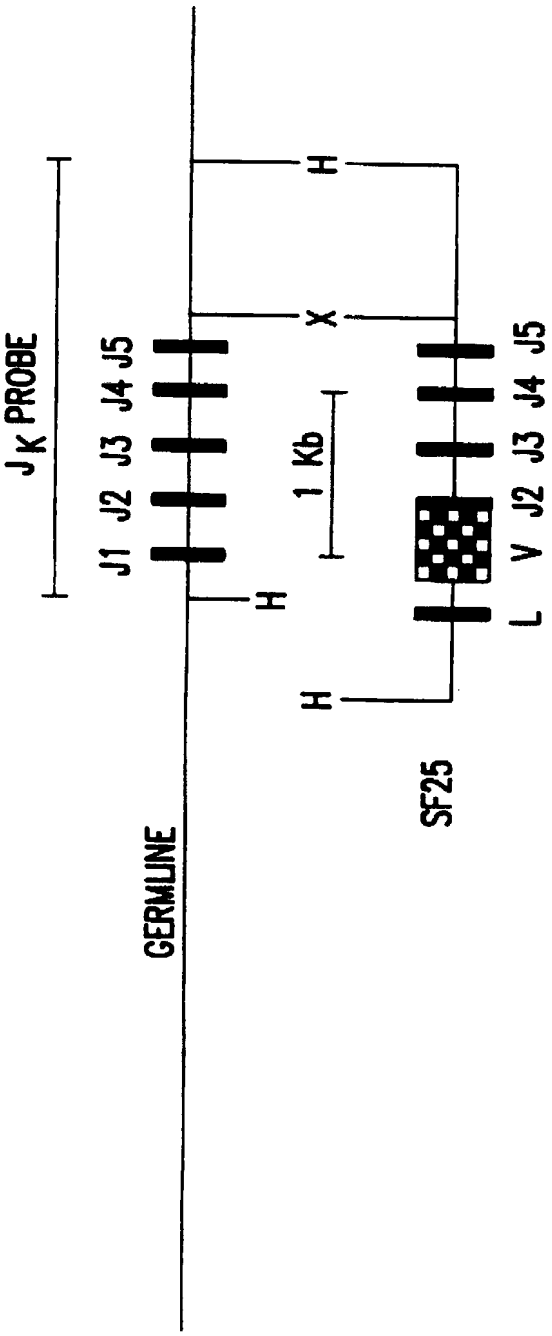
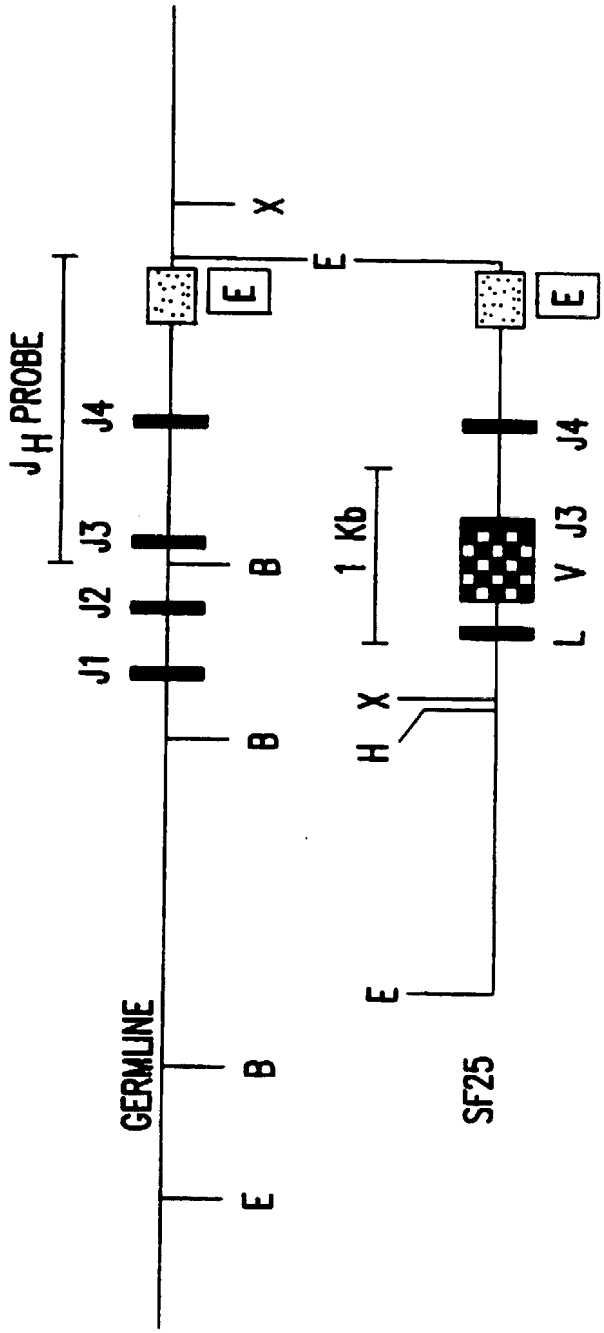

J_K PROBE
J1 J2 J3 J4 J5
H
X
H
1 Kb
H
GERMLINE
L V J2 J3 J4 J5
SF25

FIG.1B


X
E
E
E
E
J_H PROBE
J4
J3
J2
J1
B
B
1 Kb
H X
E
GERMLINE
B
E
L V J3 J4
SF25

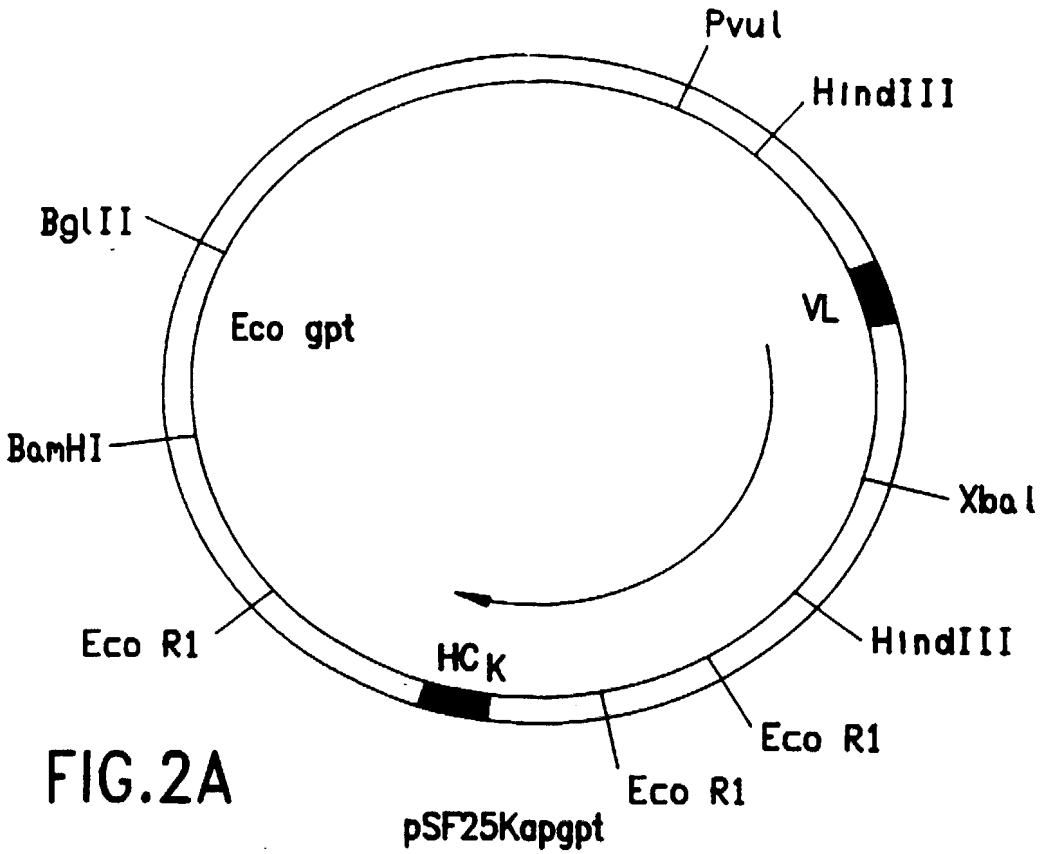

Pvul
HindIII
BglII
Eco gpt
VL
BamHI
Xbal
Eco R1
HindIII
HCK
Eco R1
Eco R1
pSF25Kapgpt

FIG.2A

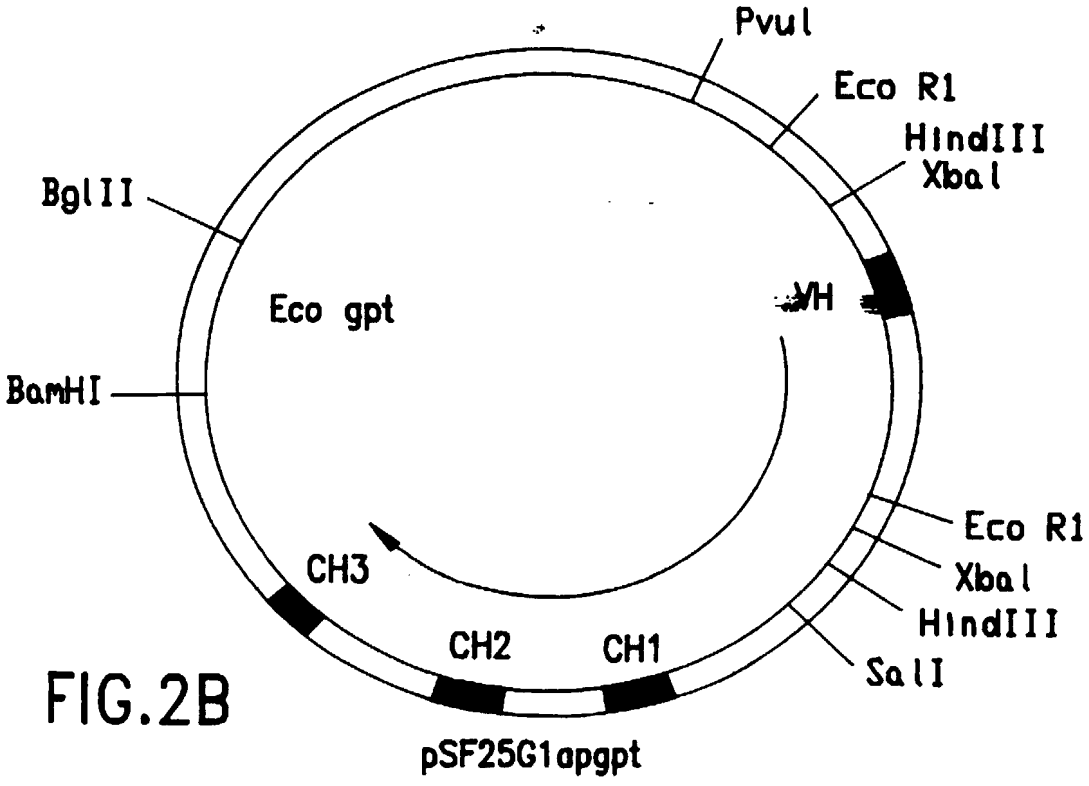

Pvul
Eco R1
HindIII
Xbal
BglII
Eco gpt
VH
BamHI
Eco R1
Xbal
HindIII
CH3
CH2
CH1
Sall
pSF25G1apgpt

FIG.2B

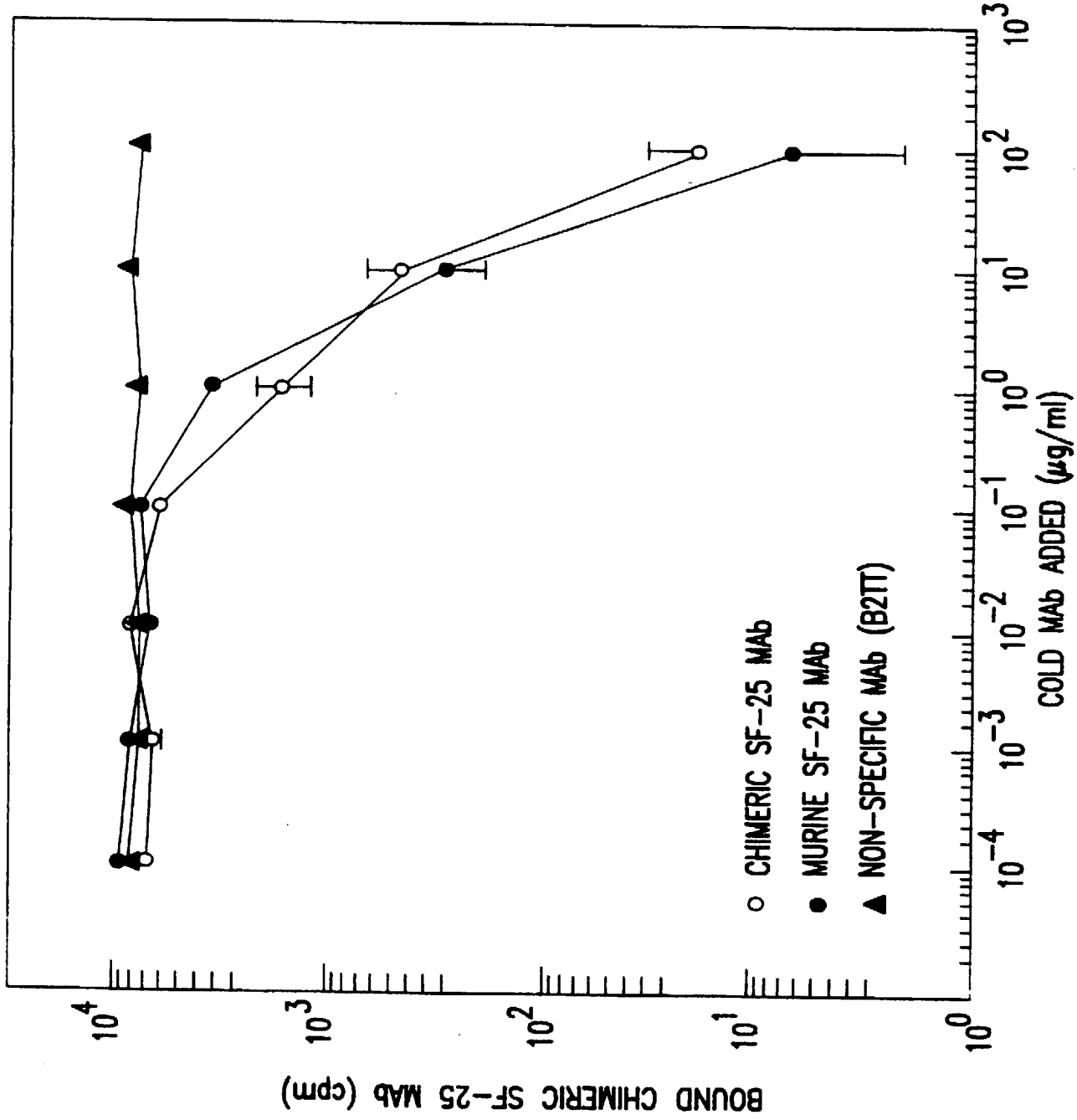

BOUND CHIMERIC SF-25 MAb (cpm)
10^0
10^1
10^2
10^3
10^4
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
10^2
10^3
COLD MAb ADDED (μg/ml)
○ CHIMERIC SF-25 MAb
● MURINE SF-25 MAb
▲ NON-SPECIFIC MAb (B2TT)

FIG.3

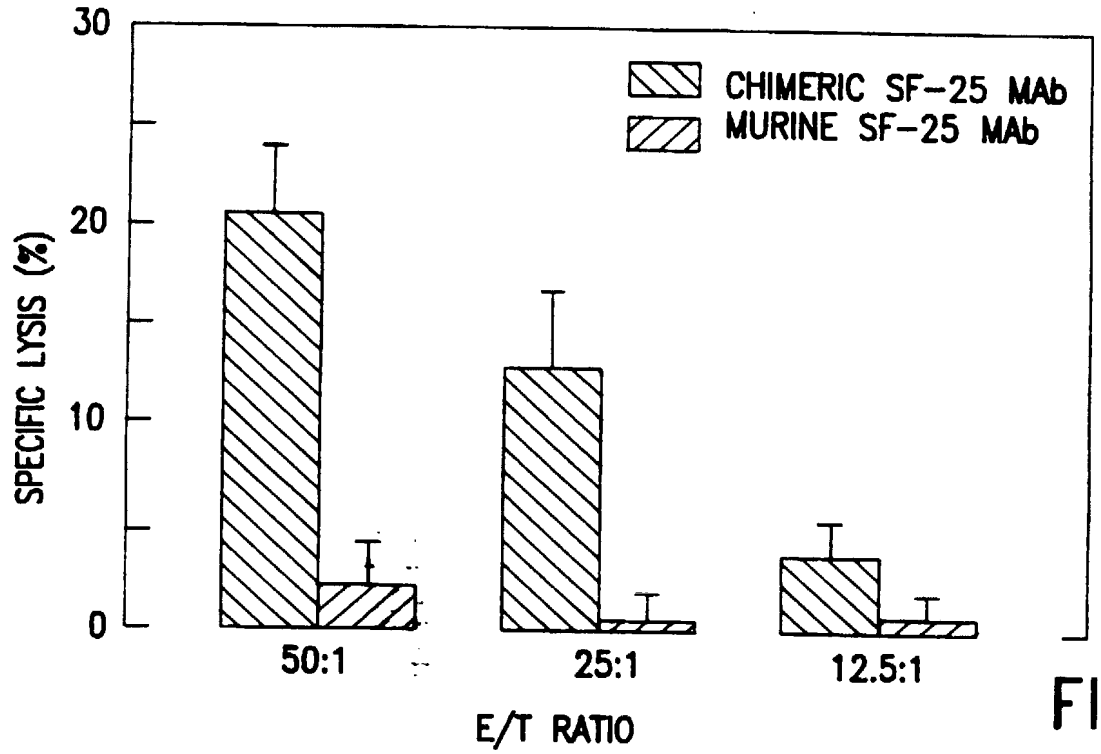

30
20
10
0
SPECIFIC LYSIS (%)
CHIMERIC SF-25 MAb
MURINE SF-25 MAb
50:1
25:1
12.5:1
E/T RATIO

FIG.4A

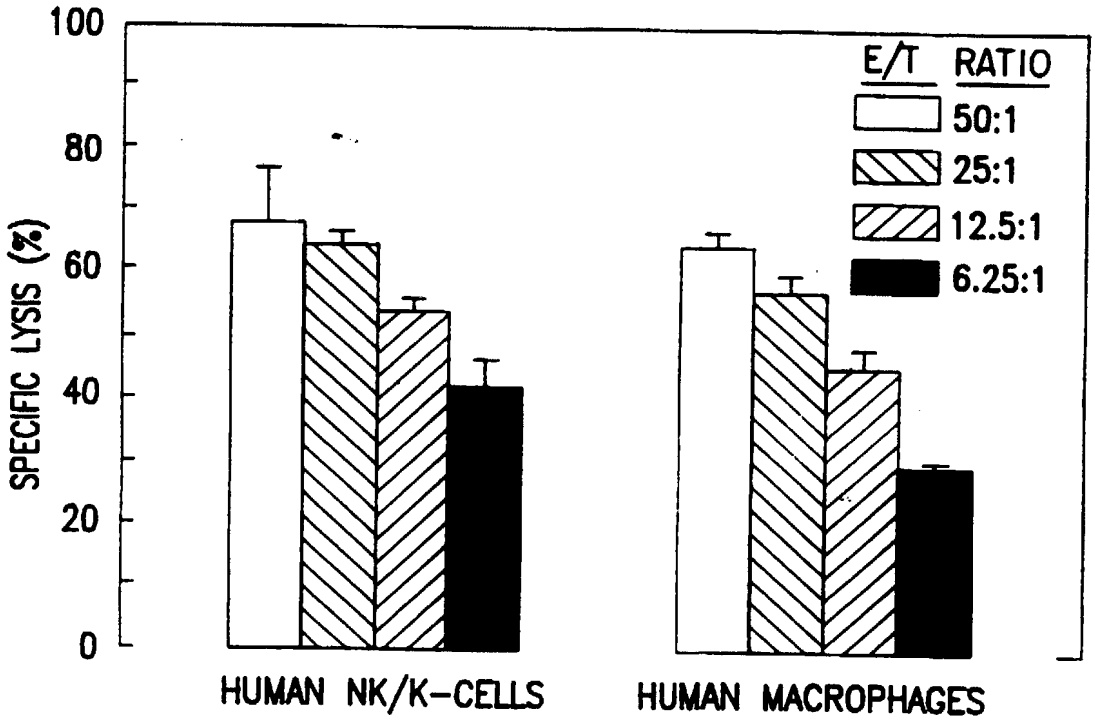

100
80
60
40
20
0
SPECIFIC LYSIS (%)
E/T RATIO
50:1
25:1
12.5:1
6.25:1
HUMAN NK/K-CELLS
HUMAN MACROPHAGES

FIG.4B

FIG.5A

FIG.5B

FIG.5C

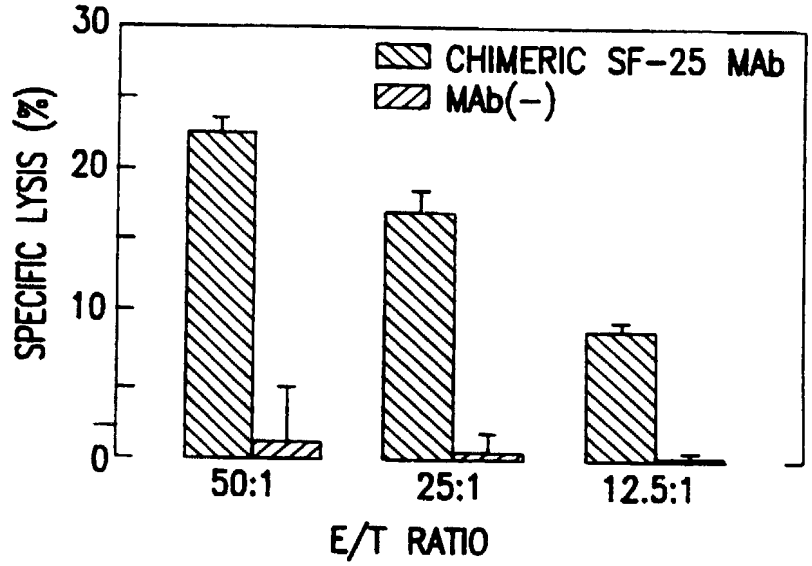


FIG.6A

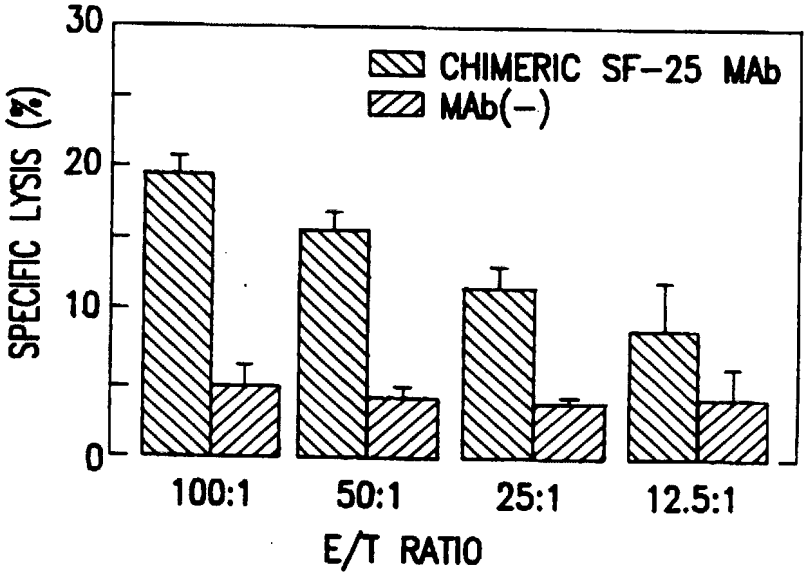


FIG.6B

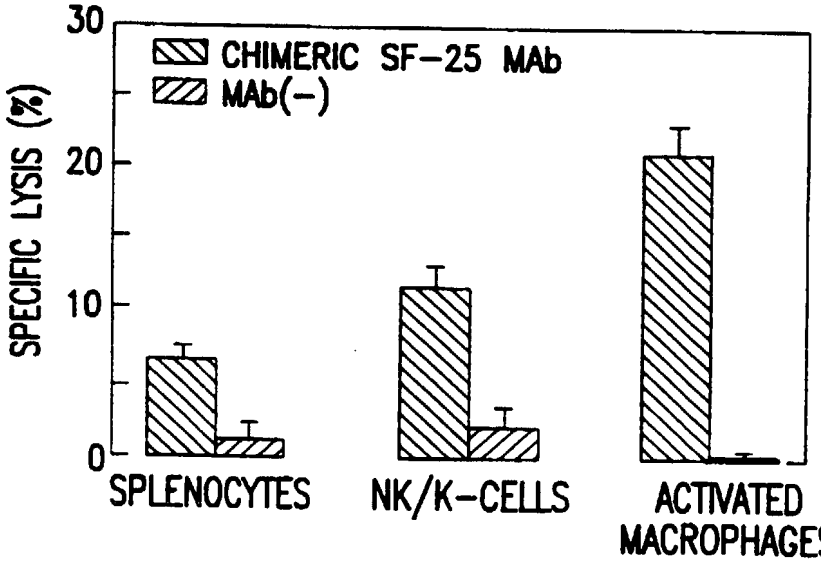


FIG.6C

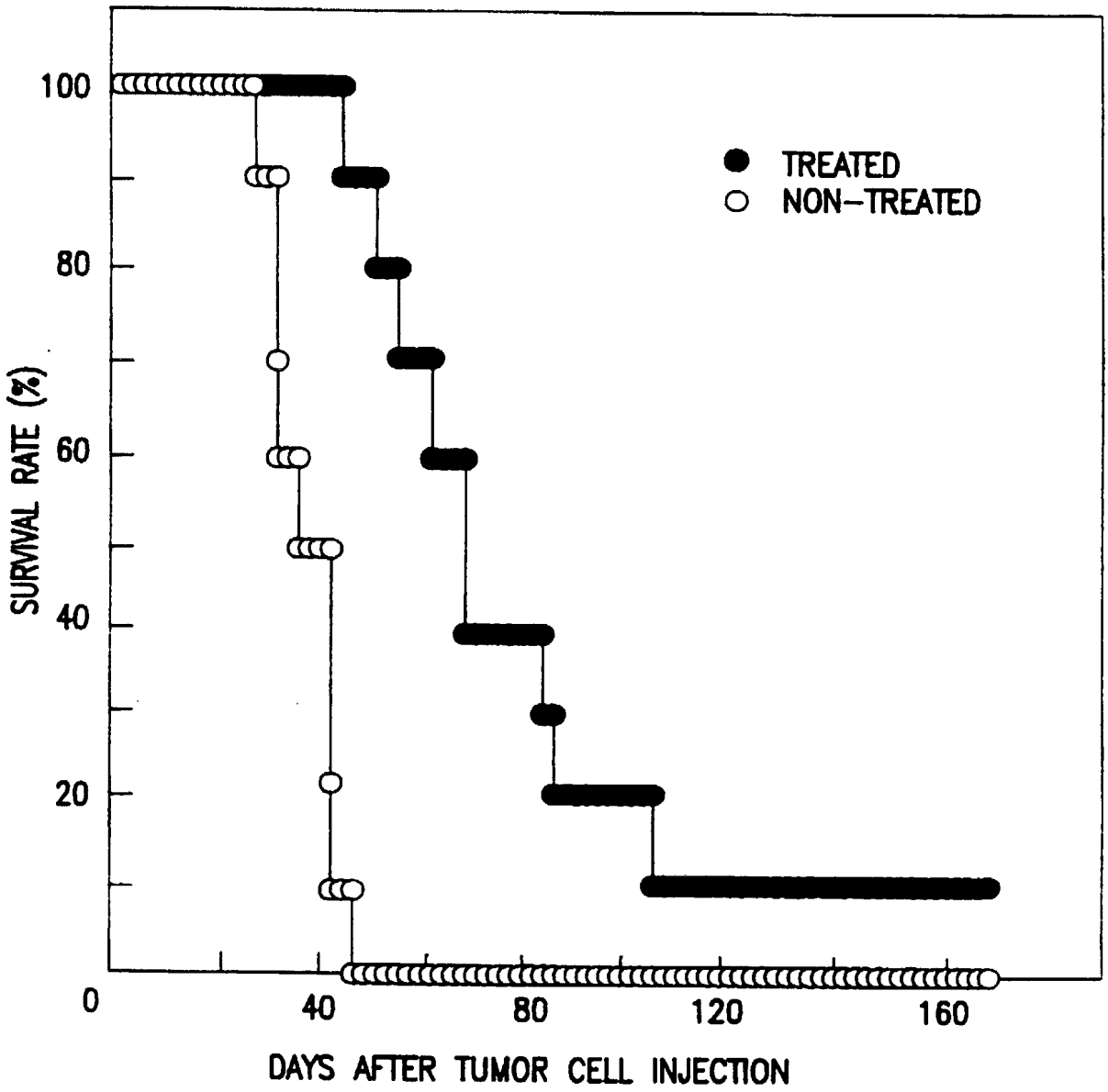

100
80
60
40
20
0
SURVIVAL RATE (%)
TREATED
NON-TREATED
40
80
120
160
DAYS AFTER TUMOR CELL INJECTION

FIG.7

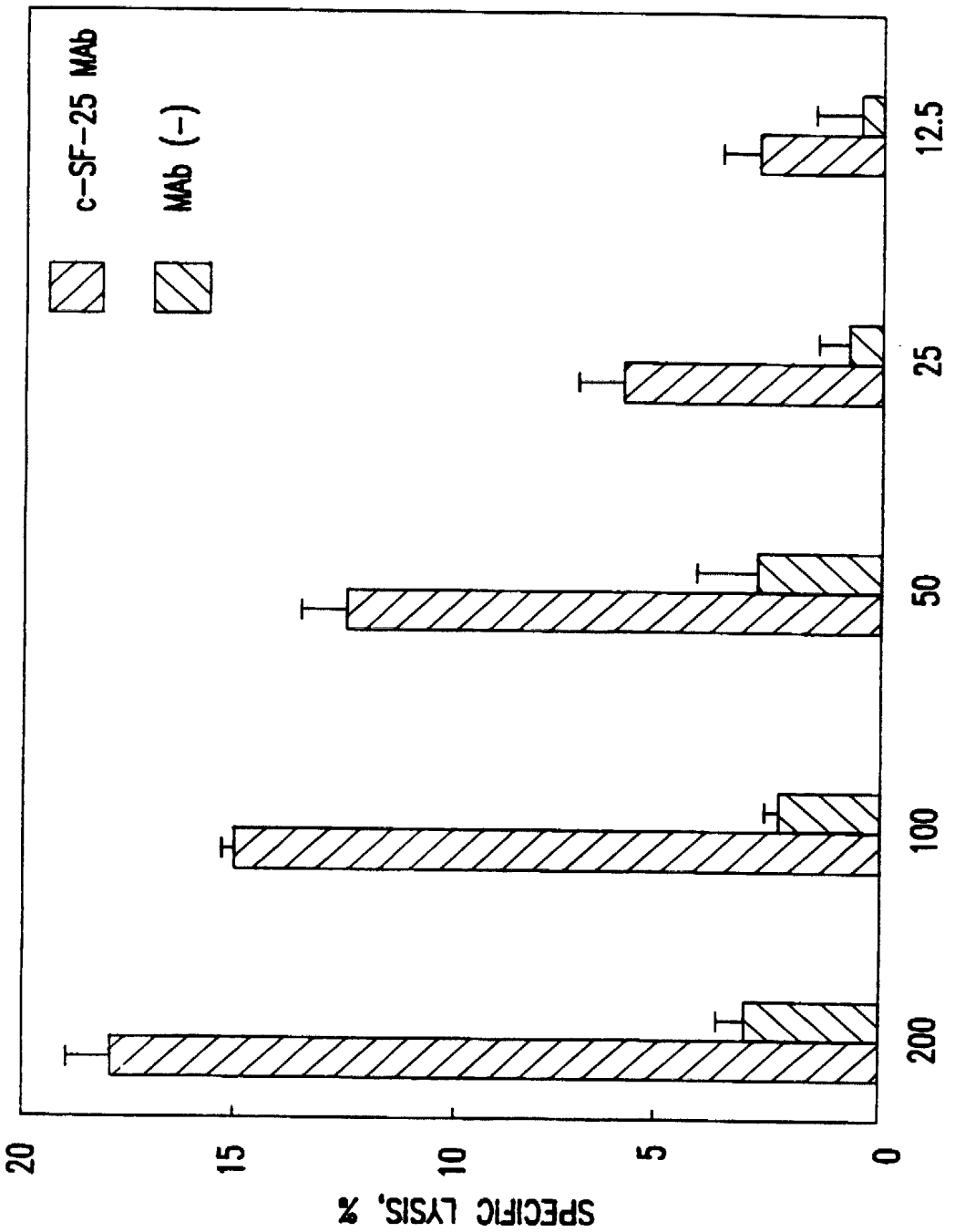

c-SF-25 MAb
MAb (-)
SPECIFIC LYSIS, %
0
5
10
15
20
E/T RATIO
12.5
25
50
100
200

FIG.8

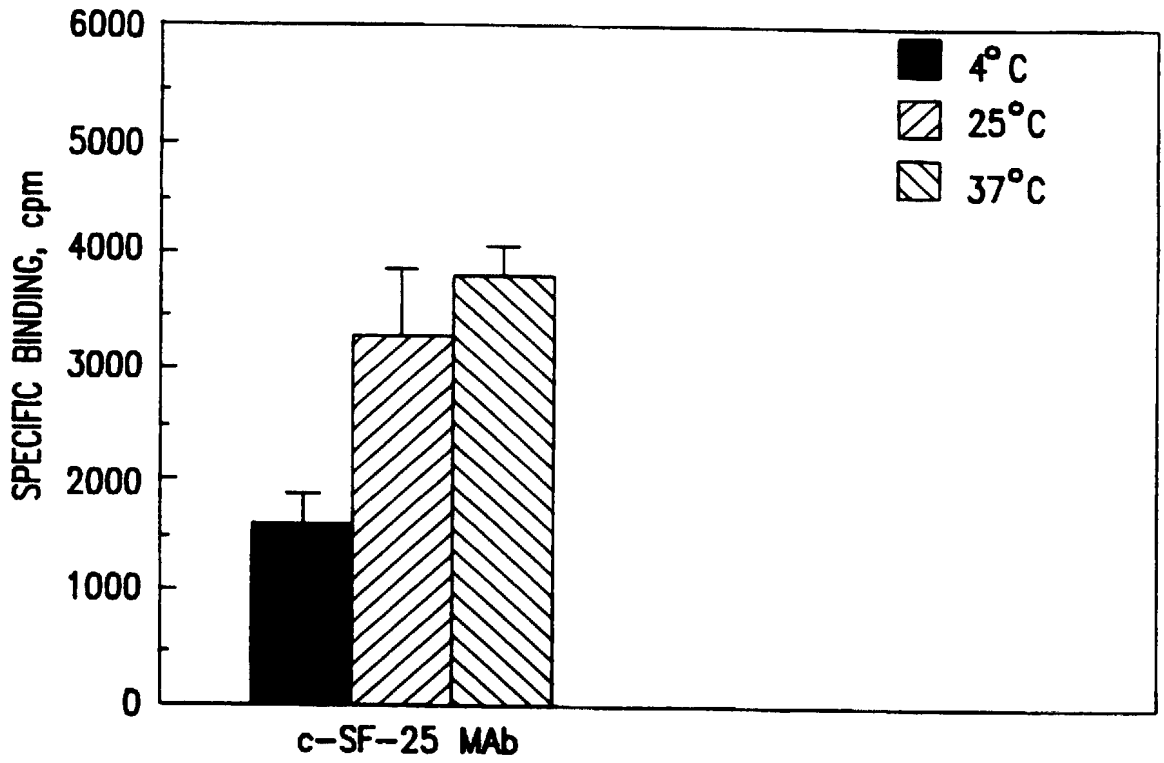

6000
5000
4000
3000
2000
1000
0
SPECIFIC BINDING, cpm
4°C
25°C
37°C
c-SF-25 MAb

FIG.9

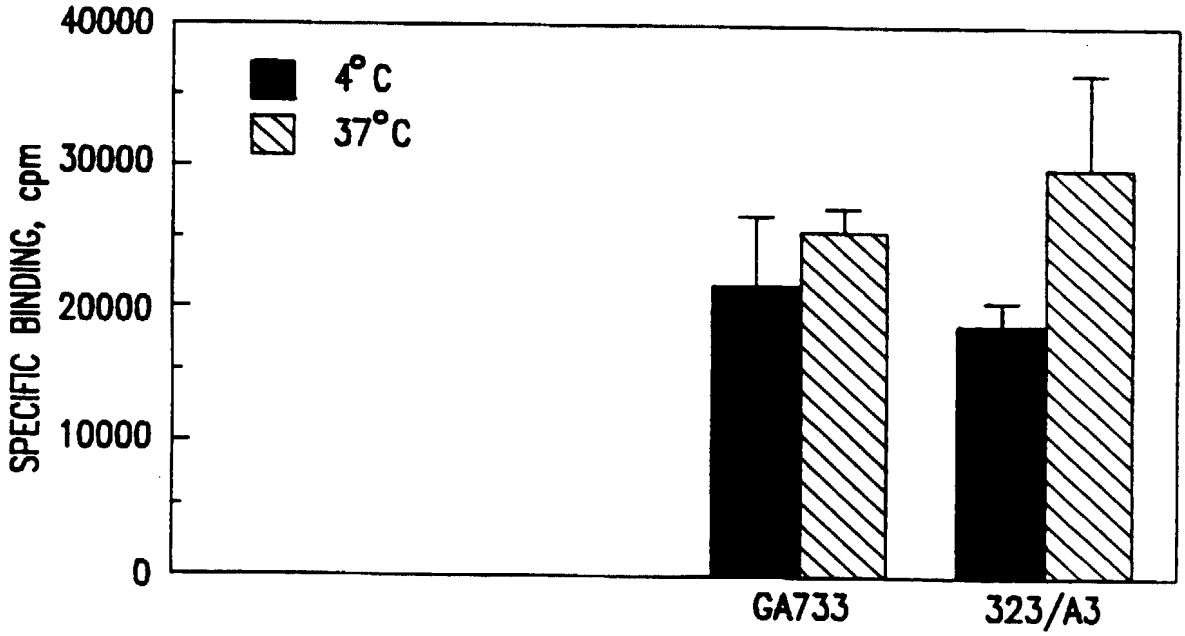

40000
30000
20000
10000
0
SPECIFIC BINDING, cpm
4°C
37°C
GA733
323/A3

FIG.10

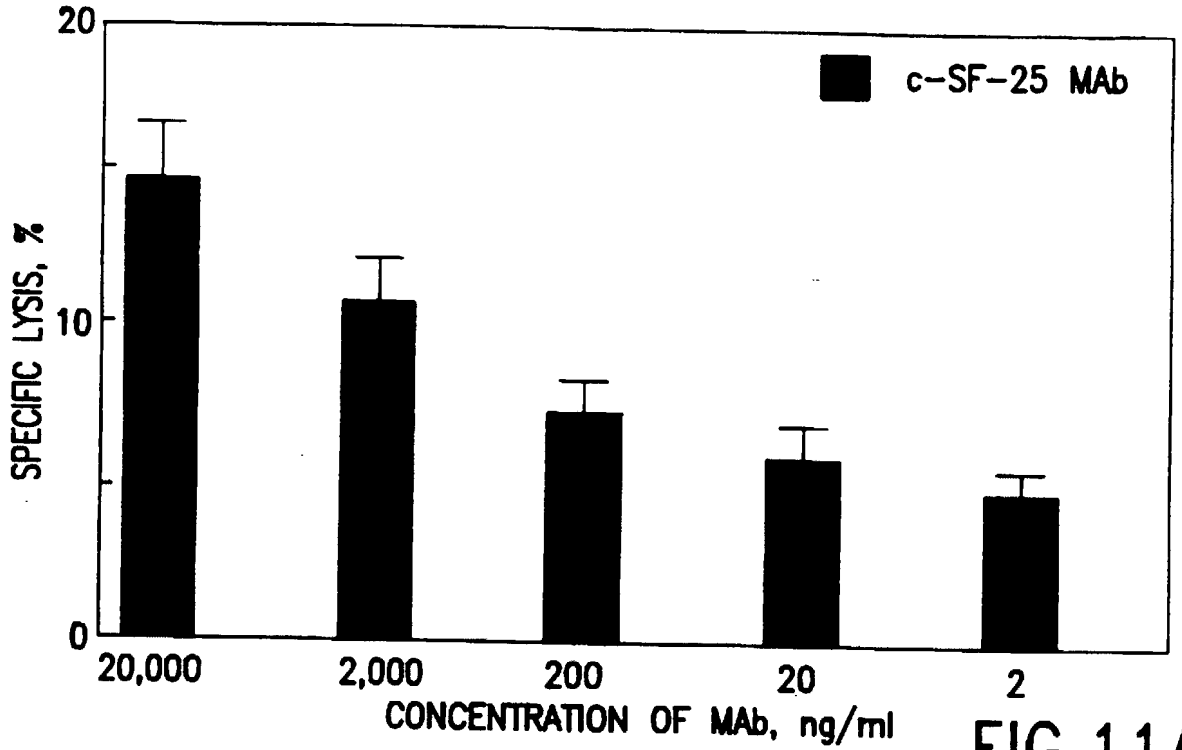


FIG.11A

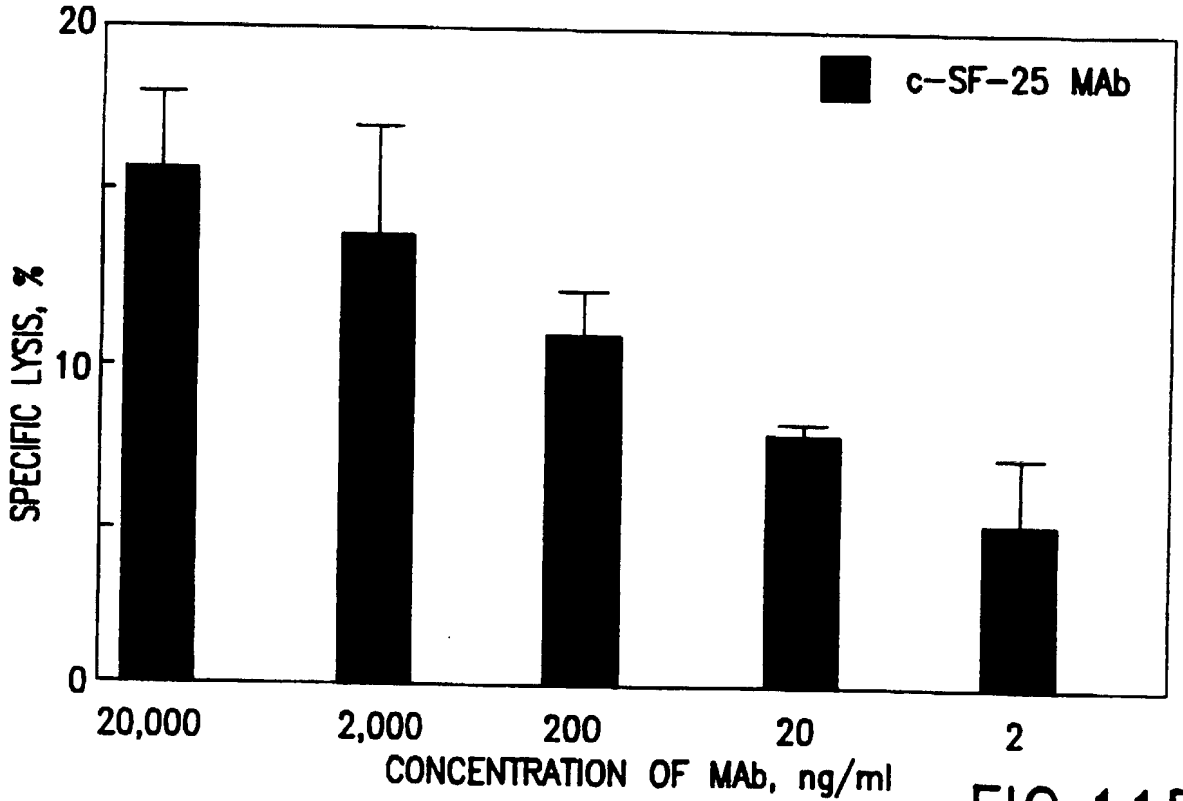


FIG.11B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08109

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/27; 435/320.1, 240.2, 252.2, 7.23, 70.21, 172.2; 530/387.3, 388.25, 391.1, 391.7; 424/1.1, 85.8, 85.91

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Cas Online, APS, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	Gastroenterology, Volume 96, No.5, Part 1, issued 1989, H. Takahashi et al., "Radioimmunolocalization of Hepatic and Pulmonary Metastasis of Human Colon Adenocarcinoma", pages 1317-29, especially page 1317.	1-65, 74-81
Y	Annals New York Academy of Sciences, Volume 507, issued 1988, S. L. Morrison et al., "Genetically Engineered Antibody Molecules and Their Application" pages 187-198, see entire document.	1-65, 74-81
Y	Methods in Enzymology, Volume 178, issued 1989, M. Better et al., "Expression of Engineered Antibodies and Antibody Fragments in Microorganisms", pages 476-496, see entire document.	8-65, 74-81
Y	Proceedings of the National Academy of Sciences USA, Volume 86, issued December 1989, C. Queen et al., "A Humanized Antibody that Binds to the Interleukin 2 Receptor", pages 10029-10033, see especially pages 10029-10031.	12-65, 74-81

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
03 December 1992

Date of mailing of the international search report
17 DEC 1992

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PAULA HUTZELL
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

BNSDOCID: <WO_____9308117A1_IA>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08109

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	Biotechnology, Volume 9, issued February 1991, G. T. Davis et al., "Single Chain Antibody (SCA) Encoding Genes: One Step Construction and Expression in Eukaryotic Cells", pages 165-169, see entire document.	54, 63, 71, 79
Y	A. Pincera et al., "Monoclonal Antibodies '84: Biological and Clinical Applications", published 1985 by Editrice Kurtis s.r.l. see pages 475-506, especially pages 475-486.	23, 25-31, 40-65, 74-81
Y	Clinical Chemistry, Volume 27 No. 11, issued 1981, E. D. Sevier et al., "Monoclonal Antibodies in Clinical Immunology" pages 1797-1806, see especially pages 1800-1802.	15-19, 21, 22, 24, 39, 43
Y	US, A, 4,735,210 (Goldenberg) 05 April 1988, col. 14-15.	20, 39-45
Y	Vogel et al., "Immunoconjugates. Antibody Conjugates in Radioimaging and Therapy of Cancer", published 1987 by Oxford University Press, see pages 259-280.	17, 19, 25-27, 29, 39-65, 74-81

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08109

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07H 15/12; C12N 15/00, 5/10, 1/20, 15/02; C07K 15/28; C12P 21/08; A61K 43/00, 39/395; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/27; 435/320.1, 240.2, 252.2, 7.23, 70.21, 172.2; 530/387.3, 388.25, 391.1, 391.7; 424/1.1, 85.8, 85.91